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The influence of schistosomiasis on the serum concentrations of retinol and retinol binding protein of a rural population in Liberia

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Summary

121 persons from rural areas of central Liberia were examined for possible interactions between helminthic infections and the retinol (vitamin A) and retinol binding protein (= RBP) status. About 2/3 of this mainly adult population had retinol and RBP serum concentrations $\leq 400 \mu\text{g/l}$ and $\leq 40 \mu\text{g/ml}$, respectively. Only one person had a serum retinol concentration $< 100 \mu\text{g/l}$ which is critical for the appearance of xerophthalmia. The retinol and RBP concentrations showed a linear, positive correlation. 19 parasitologically helminth free individuals had mean serum retinol and RBP concentrations of $414 \mu\text{g/l}$ and $43 \mu\text{g/ml}$, respectively, while the corresponding values of 20 individuals with schistosomiasis as the only helminthic infection were $339 \mu\text{g/l}$ and $35 \mu\text{g/ml}$. 65 other persons with mixed intestinal and/or tissue helminthiasis, and 9 persons with pure *Onchocerca volvulus* infection exhibited intermediate mean concentrations. 32 persons without serological evidence of helminthic infections had higher serum concentrations of retinol and RBP than 32 individuals in whose sera at least one raised antibody titer was found in the enzyme-linked immunosorbent assay with 3 different helminthic test antigens. In addition to age, sex and pregnancy schistosomiasis might be a further risk factor of latent retinol and RBP deficiency among adults and teenagers living in a schistosomiasis endemic area.

Key words: retinol; vitamin A; retinol binding protein; schistosomiasis; *Schistosoma haematobium*; *Schistosoma mansoni*; *Onchocerca volvulus*; immunity; enzyme-linked immunosorbent assay; Liberia.

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Introduction

Retinol (vitamin A) is a fat soluble vitamin which is essential for the maintenance of the normal functions of epithelial and mucous membranes in humans. In well nourished individuals the serum concentrations of retinol and of the retinol binding protein (= RBP) are above 400 $\mu\text{g/l}$ and 40 $\mu\text{g/ml}$, respectively (WHO, 1976; De Luca et al., 1979). In retinol deficiency the reserves in the liver get exhausted and the serum concentration is between 100 and 400 $\mu\text{g/l}$. In clinically manifest retinol deficiency the serum concentration is below 100 $\mu\text{g/l}$ (WHO, 1976). In protein deficient individuals the RBP serum concentration is likely to be lower than 35 to 40 $\mu\text{g/ml}$ (De Luca et al., 1979).

Subclinical and clinical retinol deficiencies are prevalent in many tropical countries, often in association with protein deficiency, diarrhoea and with viral, bacterial and parasitic infections (Scrimshaw et al., 1968). In the rain forest area of West Africa retinol deficiency seems rare because red palm oil, which is rich in retinol and its precursors, is widely consumed. In this same area schistosomiasis, onchocerciasis and ancylostomiasis are endemic (Saladin et al., 1980; Stürchler et al., 1980). On the occasion of a field study on the epidemiology and morbidity of schistosomiasis in Liberia we wanted to find possible correlations between the serum concentrations of retinol and of RBP and the prevalence of schistosomiasis and other parasitoses in a rural population.

Study population, Materials and Methods

The field investigation took place during the dry season of 1980/81, in central Liberia where previous work had identified three different zones of schistosomiasis transmission (Saladin et al., 1980): I: no transmission, II: transmission of *Schistosoma haematobium* only, and III: transmission of both *S. haematobium* and *S. mansoni*.

From zones I, II and III the population was sampled for the schistosomiasis morbidity study (Holzer et al., 1983). These were random samples from zone II (Zeansue village) and III (Synea village). In zone I (Flehla village) the town chief indicated the individuals who were to be examined. Of Synea only pooled sera were available for retinol determination. In addition a group of out-patients consulting at the Phebe hospital in Suakoko for symptoms suggesting schistosomiasis were investigated. The data of 79 females and 42 males with an age range of 5 to 35 years were available for investigation.

The details of the clinical, laboratory and parasitological methods are described in Holzer et al. (1983) and Saladin et al. (1983). All participants completed a questionnaire and had a physical examination. Urine was filtered for eggs of *S. haematobium* and tested with paper strips for proteinuria and haematuria and with a dip-slide agar for bacteriuria. Stool was analysed with formol-ether concentration (Knight et al., 1976) for eggs of *S. mansoni* and other helminths. Skin-snips from the left iliac crest for microfilariae of *Onchocerca volvulus* were placed into normal saline solution and read after 3 and 6 hours. Hematocrit, total white blood cell count and blood eosinophilia were determined with capillary blood.

Sera were processed in Basel. The enzyme-linked immunosorbent assay (= ELISA) was used for the serodiagnosis of schistosomiasis, filariasis, toxocariasis and amebiasis (Speiser, 1982). Doubtfully positive ELISA extinction values were 0.15–0.3 for schistosomiasis, 0.3–0.5 for amebiasis, and 0.5–0.7 for filariasis and toxocariasis. The indirect immunofluorescent antibody test (= IFAT) was used for the serodiagnosis of malaria. Serum retinol concentrations were determined as

Table 1. Serum concentrations of retinol and retinol binding protein (= RBP) in the study population. Adequate retinol and RBP concentrations are $>400 \mu\text{g/l}$ and $>40 \mu\text{g/ml}$, respectively.

	Retinol in $\mu\text{g/l}$		RBP in $\mu\text{g/ml}$	
	N	mean (\pm SD)	N	mean (\pm SD)
Total population	121	379.1 (139.2)	116	38.3 (13.1)
Sex				
Females	79	362.2 ^a (138.2)	75	36.6 ^b (12.2)
Males	42	410.8 ^a (137.3)	41	41.7 ^b (14.1)
Age: full years				
5-15	13	332.3 ^c (94.7)	13	31.8 ^d (7.9)
16-25	67	371.1 (108.1)	63	37.8 (11.5)
26-35	41	407.4 ^c (185.8)	40	41.3 ^d (15.8)
Location				
Flehla (zone I)	30	470.7*		
Zeansue (zone II)	39	406.6 ^e (128.2)	37	40.4 ^f (11.0)
Synea (zone III)	39	387.1 (119.3)	39	40.2 (11.7)
Suakoko (outpatients)	43	346.8 ^e (160.6)	40	34.6 ^f (15.5)

* weighted mean of 9 pools of sera

a-a t = 1.8, 2p: n.s.

c-c t = 1.9, 2p: n.s.

e-e t = 1.9, 2p: n.s.

b-b t = 2.1, 2p<0.05

d-d t = 1.9, 2p: n.s.

f-f t = 1.9, 2p: n.s.

follows: 100 μl serum samples were taken up in n-hexane with 2% isopropanol, then high pressure liquid chromatography was performed with a LiChrosorb (E. Merck, Darmstadt) Si 60 5 μm 25 cm as fixed phase. Detection was done with UV 325 nm (Uvikon LCD 725, Kontron AG, Zürich). Quantification was carried out with planimetry of the peaks in relation to an external standard. RBP was determined by immunodiffusion assay.

For probability statements t was calculated.

Results

121 persons were investigated. None of them was severely ill at the time of examination or presented with signs of marasmus or kwashiorkor. A low hematocrit ($\leq 35\%$ in females, $\leq 38\%$ in males) was found in 27 females and in 7 males. 58 persons (48%) had eggs of *S. haematobium* in the urine, 51 persons had microhematuria (among them 41 were females), 16 had proteinuria (+ + or + + +) and 12 had bacteriuria ($> 10^6$ germs/ml). In a single stool sample of 45 persons (37%) eggs of *S. mansoni* were found. 37 persons had ancylostomiasis, 29 trichuriasis and 15 ascariasis. 54 persons (45%) were infected with *Onchocerca volvulus*.

Except for a 19-year-old girl the whole study population was reactive to *Plasmodium falciparum* antigen in the IFAT. 32 persons did not respond to the various helminthic test antigens in the ELISA.

Table 2. Serum retinol and retinol binding protein (= RBP) in *females* by pregnancy and hematocrit

	Retinol in $\mu\text{g/l}$		RBP in $\mu\text{g/ml}$	
	N	mean (\pm SD)	N	mean (\pm SD)
<i>History of pregnancy</i>				
Yes	6	318.8 (55.0)	6	33.2 (4.2)
No	73	365.8 (142.5)	69	36.8 (12.6)
<i>Hematocrit</i>				
>35%	52	378.0 (135.5)	49	37.9 (11.0)
\leq 35%	27	331.8 (140.7)	26	33.8 (14.0)

Table 3. Serum concentrations of retinol and retinol binding protein (= RBP) in relation to *parasitological* findings

	Retinol in $\mu\text{g/l}$		RBP in $\mu\text{g/ml}$	
	N	mean (\pm SD)	N	mean (\pm SD)
<i>Helminth free persons</i>	19	413.8 ^{a,b} (147.1)	19	43.0 ^{c,d} (14.2)
<i>All with helminths</i>	102	369.7 ^a (130.5)	97	37.1 ^c (12.0)
<i>Single helminthiases</i>				
Schistosomiasis	20	339.0 ^b (137.9)	20	34.5 ^d (10.9)
Onchocerciasis	9	394.0 (151.6)	9	37.0 (14.9)
Intestinal nematodes	8	370.8 (109.6)	7	34.9 (14.6)
<i>Multiple helminthiases</i> *	65	375.2 (128.9)	61	38.2 (11.8)

* *Schistosoma* sp. plus *O. volvulus* plus intestinal nematodes: 21

Schistosoma sp. plus intestinal nematodes: 20

Schistosoma sp. plus *O. volvulus*: 14

O. volvulus plus intestinal nematodes: 10

a-a t = 1.2, p: n.s.

b-b t = 1.6, 2p: n.s.

c-c t = 1.7, 2p: n.s.

d-d t = 2.6, 2p < 0.02

Retinol and retinol binding protein (= RBP) by sex, age and village (Table 1). Serum retinol concentrations were $\leq 400 \mu\text{g/l}$ in 77 persons (64%), those of RBP were $\leq 40 \mu\text{g/ml}$ in 73 (63%). The lowest retinol concentration (72 $\mu\text{g/l}$) was observed in a woman consulting at Suakoko for intestinal schistosomiasis. Females had lower mean retinol and RBP concentrations than males. Pregnant females had lower values than non-pregnant females (Table 2). Adults had a better retinol and RBP status than children and teenagers. Schistosomiasis outpatients and the persons from Synea (zone III) had mean retinol serum concentrations < 400 $\mu\text{g/l}$.

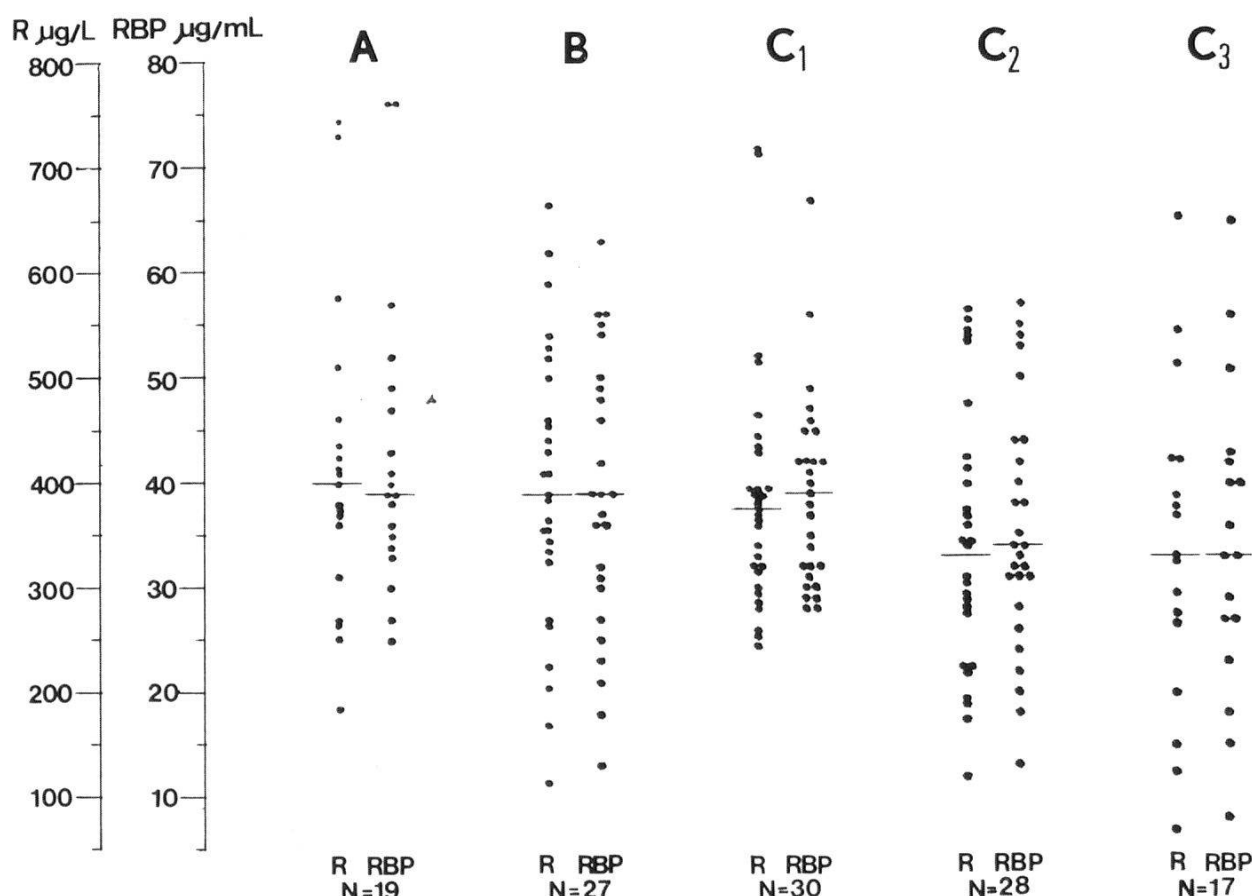


Fig. 1. Scatterdiagram of the serum concentrations of retinol (= R) and of retinol binding protein (= RBP), by parasitological findings. A = helminth free subjects; B = *Schistosoma* free subjects; C = *Schistosoma*-infected subjects. C₁: with *S. haematobium* only; C₂: with *S. haematobium* and *S. mansoni*; C₃: with *S. mansoni* only. Vertical traces indicate medians.

Retinol, RBP and biochemical results. Males and females (Table 2) with low hematocrits had lower mean retinol and RBP concentrations than their mates with adequate hematocrits. The presence of neither proteinuria nor bacteriuria seemed to influence the retinol status of the population. However, men with microhematuria had lower mean retinol and RBP concentrations (359 µg/l and 37 µg/ml, respectively) than men without hematuria (with corresponding concentrations of 418 µg/l and 42 µg/ml, respectively).

Retinol, RBP and parasitological results (Table 3, Fig. 1). Helminth free individuals (11 females, 8 males) had the highest mean retinol and RBP concentrations and persons with pure schistosomiasis had the lowest. Persons with pure onchocerciasis or with polyparasitism had intermediate values. 5 persons (4 females, 1 male) with pure *S. haematobium* infection had mean retinol and RBP concentrations of 422 µg/l and 43 µg/ml, respectively. 5 other persons (again 4 females, 1 male) with pure *S. mansoni* infection had corresponding values of 353 µg/l and 34 µg/ml, respectively. 10 of 19 helminth free persons, and 16 of 20 with pure schistosomiasis had retinol concentrations <400 µg/l.

Table 4. Serum concentrations of retinol and retinol binding protein (= RBP), and the presence of *anti-helminthic antibodies* as measured by the enzyme-linked immunosorbent assay (= ELISA)

	Retinol in $\mu\text{g/l}$		RBP in $\mu\text{g/ml}$	
	N	mean (\pm SD)	N	mean (\pm SD)
<i>No anti-helminthic antibodies</i>	32	425.7 ^{a,c} (140.7)	29	42.2 ^{b,d} (13.1)
<i>All with anti-helminthic antibodies</i>				
Only doubtful reactions*	6	361.8 (84.1)	6	36.3 (6.8)
At least one strong reaction*	32	321.9 ^a (101.4)	31	35.2 ^b (11.2)
<i>Anti-schistosomal antibodies only</i>				
Doubtful and strong reactions*	35	371.8 (146.1)	35	36.8 (14.1)
Only strong reaction*	12	339.3 ^c (147.6)	12	33.8 ^d (13.8)
<i>Anti-filarial and/or anti-toxocaral antibodies only</i>				
Doubtful and strong reactions*	16	403.7 (131.4)	15	37.5 (13.9)
Only strong reaction*	8	351.6 (106.8)	5	36.8 (6.1)

* For cut-off points see Materials and Methods

a-a t = 3.4, 2p < 0.002

b-b t = 2.3, 2p < 0.05

c-c t = 1.8, 2p: n.s.

d-d t = 1.8, 2p: n.s.

Retinol, RBP and serological results (Table 4). Persons without anti-helminthic antibodies demonstrable in ELISA had higher mean retinol and RBP concentrations than persons whose sera gave a positive ELISA reaction with one or more helminthic test antigens. When among the 32 non-responders (19 females, 14 males) those (11 subjects) with parasitologically demonstrated *Schistosoma* infection were removed, the mean retinol and RBP concentrations in the remaining 21 persons without serological or parasitological evidence of schistosomiasis rose to $453 \pm 136 \mu\text{g/l}$ and $44 \pm 14 \mu\text{g/ml}$, respectively. 8 persons with neither parasitological nor serological evidence of any helminthiasis had mean retinol and RBP concentrations of $479 \mu\text{g/l}$ and $49 \mu\text{g/ml}$, respectively.

11 persons exhibited antibodies against *Entamoeba histolytica* in ELISA (extinction ≥ 0.3). They had mean retinol and RBP values of $276 \mu\text{g/l}$ and $25 \mu\text{g/ml}$, respectively.

The relation of retinol and RBP. Within the range of observed values retinol and RBP showed a linear, positive correlation ($r = 0.6$, $p < 0.001$).

Discussion

This study forms part of an investigation of various aspects of schistosomiasis in central Liberia (Saladin et al., 1980; Saladin et al., 1983; Holzer et al., 1983). The sampling procedure and the physical examination were designed for symptoms and signs caused by schistosomiasis, not by retinol deficiency.

However, general nutritional aspects were regarded including the measurement of height and weight.

The population at risk of retinol deficiency are mainly pre-school children in tropical countries (Oomen et al., 1964; WHO, 1976; Sommer et al., 1981; WHO, 1982). Mechanisms leading to retinol deficiency are: a) inadequate composition or quantity of the diet, b) inadequate storing or cooking of food stuffs (Bauernfeind, 1978), c) impaired conversion of retinol equivalents to retinol within the intestinal tract, d) malabsorption of retinol, particularly when diarrhea or intestinal parasitoses such as ascariidiasis are present, and e) non-delivery to the periphery because the retinol binding protein (= RBP) is deficient (WHO, 1976; De Luca, 1978; De Luca et al., 1979).

In Africa retinol deficiency is more frequent and more often manifests itself clinically in the dry and savannah regions than in the rain forest area, because in the latter the consumption of red palm oil and mango fruits prevents deficiency (Oomen et al., 1964; Le François et al., 1980). The staple food in central Liberia is rice with red palm oil sauce and leafy vegetables. Fruits, fish, shrimps and meat are eaten occasionally. 100 g of raw mangos contain 190 μg of retinol equivalents compared to 15,000 μg in 100 g of red palm oil (Périssé and Polacchi, 1979). It is, therefore, not surprising that only 1 individual had a critically low serum retinol concentration and that signs of clinically manifest deficiency were not found. However, about $\frac{2}{3}$ of the population investigated, mainly older children and adults, had biochemical signs of both retinol and RBP deficiency. Lauber and Haller (1980) made similar findings among children in the rain forest of the Ivory Coast. It is possible that the biochemical criteria of retinol deficiency have to be re-evaluated for some tropical areas. It is, however, more likely, that one of the above mentioned deficiency mechanisms is responsible for the discrepancy between a seemingly adequate diet and the biochemically inadequate retinol and RBP status observed.

Here intestinal infections come into play. The interactions between retinol, the host's immune response and viral, bacterial and parasitic infections are multi-directional and complex (Scrimshaw et al., 1968; Lee and Aboko-Cole, 1979; Glazebrook and Davis, 1979; Beisel et al., 1981; WHO, 1982). In retinol deficient persons the impaired epithelial barrier might facilitate the penetration of helminth larvae through the skin or the intestinal mucosa. Such a mechanism has been demonstrated in experimentally infected retinol deficient animals (Darip et al., 1979). Like in experimental conditions (Zile et al., 1979; Nauss et al., 1979) in retinol deficient persons the cellular immunity might be depressed. On the other hand intestinal parasites may impair the absorption of retinol. Our own preliminary investigations (Stürchler et al., 1981) point to the possibility that growing helminth larvae and reproductive adult worms might need some of the host's retinol.

The epidemiological, parasitological and serological results of this study are all in favour of an inverse correlation between schistosomiasis on the one

hand and the retinol and RBP status on the other hand. However, this field study does not sort out the contribution of the various parasite species found with certainty. Also, those persons without serological and/or parasitological evidence of schistosomiasis or other helminthiasis and with a better biochemical retinol and RBP status than the infected ones might differ from the rest of the population by a better socioeconomical status and by better nutrition. Other studies gave contradictory results. Haller and Lauber (1980) found a correlation of low retinol serum concentrations with onchocerciasis and ascariidiasis, but not with schistosomiasis, in children of the Ivory Coast, while Mansour et al. (1979) observed lower retinol serum concentrations in adult Egyptians with variously severe forms of intestinal schistosomiasis with or without chronic salmonellosis than in uninfected controls.

A causal relationship could only be demonstrated with certainty by animal experiments or by studying the in vitro retinol uptake of adult schistosomes.

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