

Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 44 (1987)
Heft: 2: A longitudinal study in a rural Tanzanian community 1982-1984

Artikel: A longitudinal study on relations of retinol with parasitic infections and the immune response in children of Kikwawila village, Tanzania
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DOI: <https://doi.org/10.5169/seals-313831>

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A longitudinal study on relations of retinol with parasitic infections and the immune response in children of Kikwawila village, Tanzania

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Summary

From 1982 to 1984 170 children of Kikwawila village (Kilombero district, Tanzania) were followed for nutritional (anthropometric measures, hematocrit, serum retinol, prealbumin, and zinc concentrations), parasitological (malaria parasitemia, urinary schistosomiasis, intestinal parasites) and immunological characteristics. Between 2.9% and 12.4% had serum retinol levels <100 µg/l which indicate deficiency. Retinol concentrations were correlated with age, hematocrits, prealbumin levels and mid upperarm circumferences. The latter correlation may be useful in nutritional surveys and primary health care programs for the identification of populations at risk of retinol deficiency. No association was found between average retinol levels and the presence of parasites, with the exception of malaria. Retinol levels were inversely correlated with malaria parasitemia in 1982, and directly correlated with antibody titers to synthetic sporozoite peptide in 1984. Since retinol, malaria parasitemia, and anti-sporozoite antibodies increased with age, confounding by age could not be excluded. Six months after administration of ornidazole in a single oral dose of 10 mg/kg, a significant effect on the prevalence of *Giardia lamblia* was found. Following treatment, average retinol levels were increased in persons with confirmed *G. lamblia* infections, but not in uninfected or untreated controls.

Key words: retinol; prealbumin; mid upperarm circumference; malaria; *Giardia lamblia*; ornidazole.

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Introduction

Xerophthalmia is prevalent in many developing countries, particularly in preschool children (Sommer et al., 1981 and 1983). It is often associated with protein energy malnutrition, diarrhea or measles. In animal experiments retinol deficiency produces cellular depletion of lymphatic organs and marked reduction of cellular immunity. Retinol is a component of natural resistance and has been termed an antiinfective vitamin (De Luca et al., 1979; Beisel, 1982).

Giardia lamblia, *Ascaris lumbricoides* and *Strongyloides stercoralis* can impair absorption of fat and retinol (Mahalanabis et al., 1976 and 1979; Tomkins, 1979; Rosenberg and Bowman, 1984), particularly in patients with a high parasite load (Brown et al., 1980). Low serum retinol levels have been found in persons with intestinal schistosomiasis (Mikhail and Mansour, 1982; Stürchler et al., 1983). Nutritional implications of malaria have been critically reviewed by McGregor (1982). In hyperendemic areas malaria predisposes to low birth weight, protein energy malnutrition, and anemia. Nutritional factors which reduce the risk of malaria morbidity include iron deficiency (Murray et al., 1975 and 1978a; Oppenheimer et al., 1984), regular milk diet (Murray et al., 1978b and 1980), and riboflavin deficiency (Thurnham et al., 1983).

Some of our research efforts are devoted to the relations of nutritional status with parasitic infections and immune response in humans (Stürchler et al., 1981, 1983, 1986a, 1986b). In Kikwawila village we had the opportunity to follow the retinol status of a rural population over three consecutive years, and to investigate relations with parasitic infections and the production of antibodies.

Population, Materials and Methods

Study area. The study was undertaken in the Kapolo and Kikwawila sectors of the village of Kikwawila situated 14 km to the northeast of Ifakara, in the Kilombero District, Morogoro Region, southeast Tanzania (Tanner et al., 1987a).

Study population. The study was part of a longitudinal community based project. All children between 1 month and 15 years of age in the Kikwawila and Kapolo sectors underwent comprehensive, repeated cross-sectional clinical, anthropometrical, biochemical, parasitological and serological examinations in October 1982, 1983 and 1984 (Tanner et al., 1987a, 1987b). Of about 600 children examined in each survey, a cohort of 170 children could be followed through all three years.

Between 1982 and 1984 mass treatments, latrine campaigns, and other health interventions were performed (Tanner et al., 1987a, 1987b). In particular, in May 1983 a major fraction of the population of Kikwawila received a single oral dose of ornidazole 40 mg/kg for treatment of *Giardia lamblia* infections.

Parasitology. Single capillary blood, stool and urine samples were collected from participants in each of the three surveys. Field-adapted parasitological standard techniques were used (Tanner et al., 1987b). After reading of ≥ 50 fields, malaria parasite densities on thick blood smears were graded from 0 to 5+: 0 = negative, 1+ = < 1 parasite/field (corresponding to < 400 parasites/ μl for an assumed leucocyte count of $0.8 \times 10^9/l$), 2+ = 1–3/field, 3+ = 4–10/field, 4+ = 11–30/field, and 5+ = > 30 /field (corresponding to > 12000 parasites/ μl).

Serology. Sera were kept frozen; in Tanzania at -20° , and after shipment to Switzerland on dry ice at -70° . Antibodies against *S. mansoni* adult worm antigens were determined with standardized ELISA assay (Speiser, 1982). Antibodies to *G. lamblia* (antigen kindly provided by Ph. D. Smith, National Institutes of Health, Bethesda, U.S.A.) and to tetanus toxoid (Te Anatoxal vaccine antigen, Schweizerisches Serum- & Impfstitut Bern, Switzerland, diluted 1:1000) were determined with the same ELISA procedure. Antibodies to the circumsporozoite (CS) protein of *Plasmodium falciparum* were determined with an ELISA by using synthetic tetrapeptide consisting of about 40 (Asn-Ala-Asn-Pro = NANP) repeats (Del Giudice et al., 1986). Sera were tested diluted 1:200. Results were read in absorbance at 492 nm and titers were expressed as log 10 of optical densities (OD).

Biochemical methods. Low quantities of sera impeded biochemical determinations on all 170 children in the cohort. Serum retinol levels were determined with high pressure liquid chromatography (Stürchler et al., 1983); values $<100 \mu\text{g/l}$ indicate deficiency (WHO, 1982). Prealbumin was measured with M-Partigen immunodiffusion plates (Behringwerke AG and Hoechst-Pharma AG, Zürich). The standard curve was established with a human control serum (Behringwerke AG) undiluted, and at dilutions 1:2 and 1:4 (Betschart et al., 1987). Zinc was determined by atomic absorption spectroscopy (Varian model with microsampling equipment); sera and an external standard were directly aspirated into the flame and read at 213.9 nm.

Data analysis. Data were analysed on a personal computer with a statistics program (StatView, BrainPower, California, U.S.A.). The following statistics were calculated: χ^2 for contingency tables, z_i for proportions and incidences, unpaired or paired t for continuous variables with quasi-normal distribution, and simple regression analysis (F value) or Sperman rank correlation (z_i value) for one dependent and one independent variable. Adjustment for age was feasible when enough numbers were in the cells. Statements on age refer to the initial age in 1982.

Results

The 170 children were followed from 1982 to 1984 and represented the study population. In a subgroup of 50 children serum retinol determinations were available for three consecutive years. In this subgroup there were significantly fewer 0–5-year-old children than in the study population, but sex ratios were the same in both groups (females:males = 3:2).

Nutritional status. Few cases of conjunctival xerosis were diagnosed on physical examination; Bitot's spots or advanced stages of xerophthalmia were not seen (Degrémont et al., 1987). In 1982, 1983, and 1984 aggregated average ($\pm\text{sd}$) serum retinol concentrations were 206 (86), 190 (86), and 244 (97) $\mu\text{g/l}$, respectively. The increase from 1983 to 1984 was significant ($t = 4.8$, $p < 0.0005$). In the same years 13 of 120 (10.8%), 21 of 170 (12.4%), and 3 of 103 (2.9%) participants had retinol concentrations $<100 \mu\text{g/l}$. Again the difference between 1983 and 1984 was significant ($t = 4.8$, $p < 0.0005$).

Retinol concentrations were comparable in males and females in all three years. Mean retinol levels increased with progressing age groups (Table 1), and retinol concentrations and age were correlated in all three years ($p < 0.0001$, <0.025 and <0.0001 , respectively, Fig. 1). In the subgroup, the same age relations were seen. In addition, a cohort effect was apparent: mean retinol values increased from 1982 to 1984, but within all cohorts concentrations temporarily decreased during 1983 (Table 2).

Table 1. Kikwawila village. Serum retinol levels in 170 children, by age groups

Age group ^a	Number, retinol, significance	Years of examination		
		1982	1983	1984
0-5	N	23	51	28
	Retinol	149.5±72.7 ^b	173.3±77.1	197.0±83.1
6-10	N	37	49	45
	Retinol	215.0±63.5	172.8±69.0	237.9±89.0
	p vs 6-10 years	<0.0005	ns	<0.05
11-15	N	27	35	28
	Retinol	276.5±75.3	231.7±96.9	298.9±100.4
	p vs 11-15 years	<0.0005	<0.005	<0.005

^a in years, according to initial age in 1982

^b µg/l: mean±sd

Table 2. Kikwawila village. Serum retinol levels in 3 age cohorts followed for 3 years (N = 50)

Age group ^a	N in cohort	Retinol (µg/l) and p	Years of examination		
			1982	1983	1984
0-5	9	Retinol	142.7±60.9 ^b	131.7±70.8	176.7±48.8
6-10	20	Retinol	219.7±73.7	162.1±54.9	247.9±90.5
		p vs 6-10 years	<0.01	ns	<0.025
11-15	21	Retinol	282.4±77.5	205.8±87.3	302.7±107.6
		p vs 11-15 years	<0.01	<0.05	<0.05

^a in years, according to initial age in 1982

^b µg/l: mean±sd

Retinol concentrations were correlated with mid upperarm circumference (MC) measures in all three years ($p < 0.0001$, < 0.025 and < 0.0001 , respectively, Fig. 2). This correlation was maintained in age groups 6-10 years, and 11-16 years. Increments of height were not correlated with retinol levels. In all three years, retinol concentrations were correlated with serum prealbumin concentrations ($p < 0.05$, < 0.05 and < 0.001 , respectively) and with hematocrits ($p < 0.0001$, < 0.025 and < 0.00025 , respectively) (data not shown). Numbers were too small to permit stratification by age. Paired zinc and retinol concentrations were associated with each other in the individual (paired t of 8.6 and 6.8 in 1983 and 1984, respectively, with $p < 0.0005$), but not in the community.

Retinol and parasitic infections. From 1982 to 1984 less than 10% of the study population remained free of five major parasitic infections (malaria,

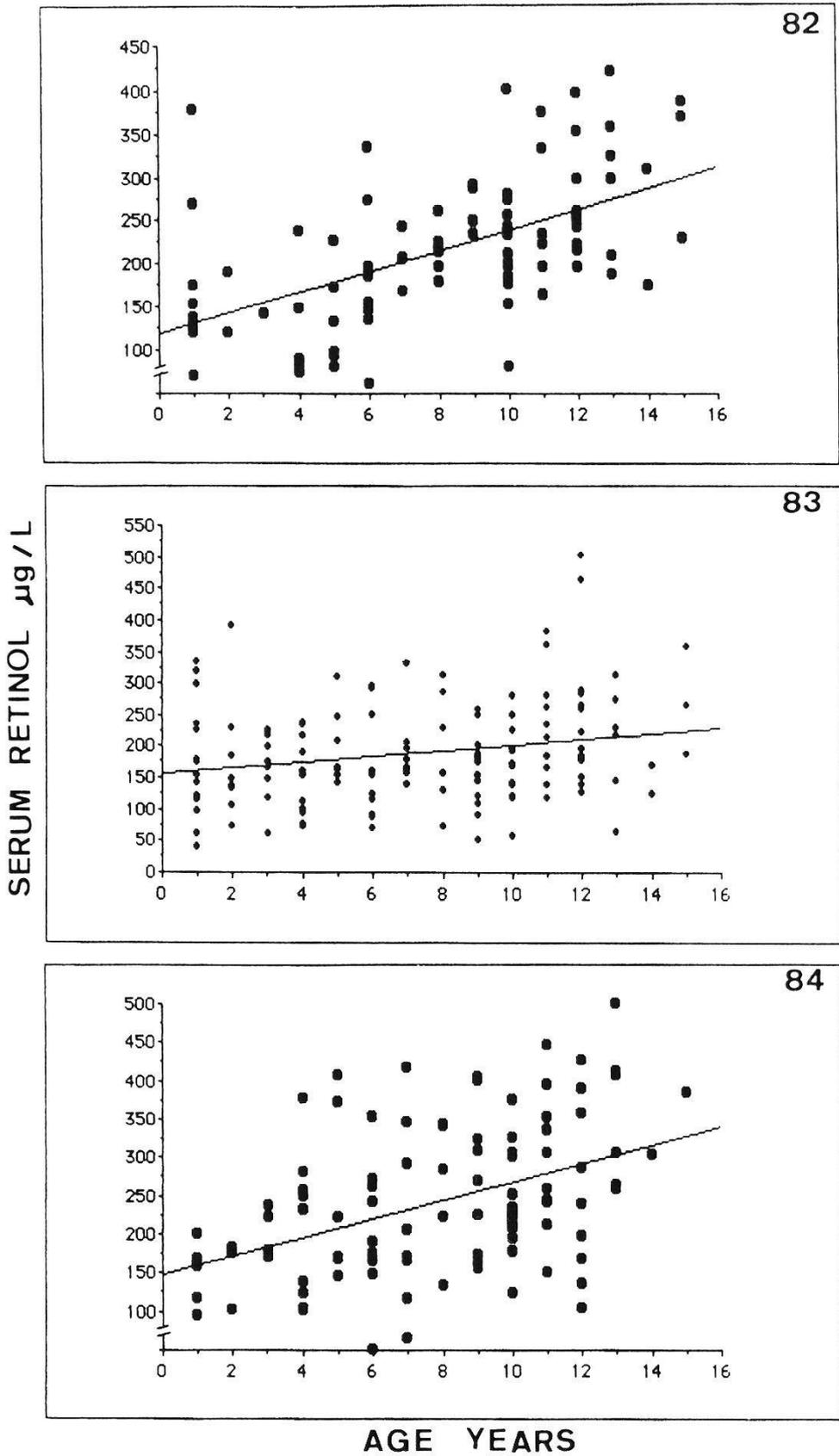


Fig. 1. Correlation of serum retinol concentrations ($\mu\text{g/l}$) of 170 children examined with age (years) for 1982 ($y = 11.8x + 120.9$; $R^2 = 0.31$, $p < 0.0001$), 1983 ($y = 4.4x + 156.5$; $R^2 = 0.05$, $p < 0.025$) and 1984 ($y = 12.1x + 148.4$; $R^2 = 0.20$, $p < 0.0001$).

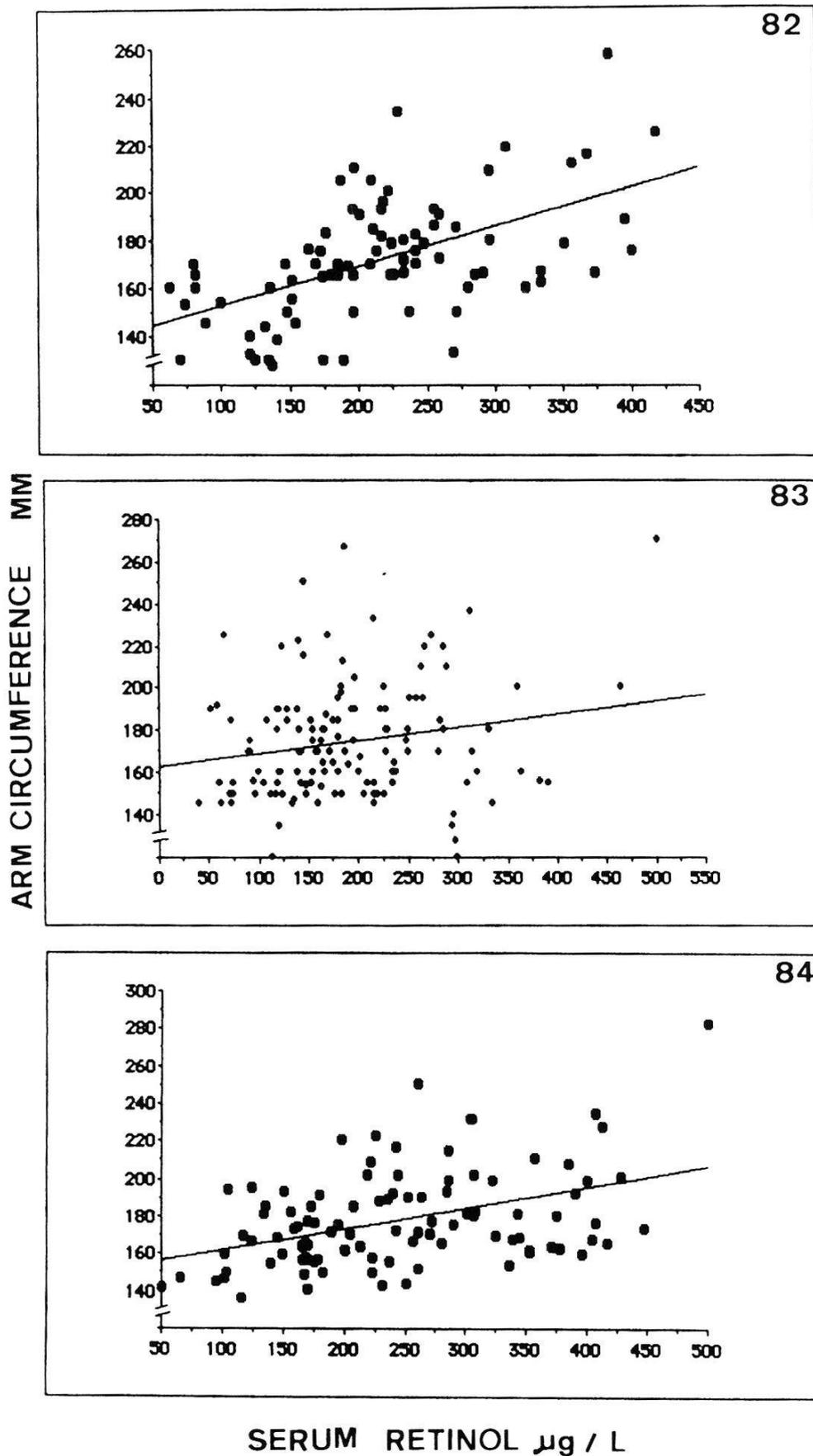


Fig. 2. Correlation of mid upperarm circumference measurements (mm) with serum retinol concentrations ($\mu\text{g/l}$) for 1982 ($y = 0.17x + 135.9$; $R^2 = 0.30$, $p < 0.0001$), 1983 ($y = 0.06x + 162.7$; $R^2 = 0.04$, $p < 0.025$) and 1984 ($y = 0.11x + 150.8$; $R^2 = 0.18$, $p < 0.0001$).

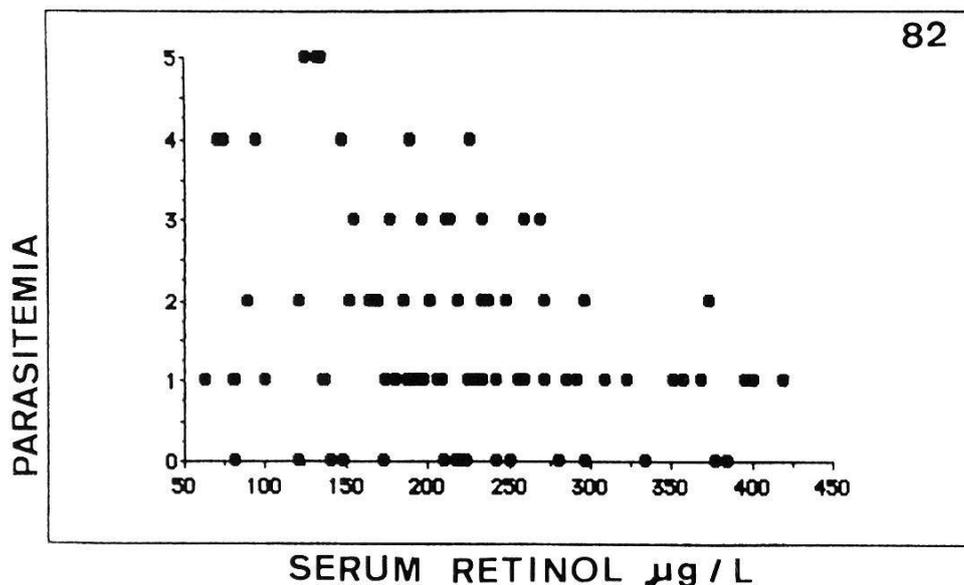


Fig. 3. Correlation of malaria parasitemia (semiquantitatively: grades 0 to 5 on thick smear) with serum retinol concentrations ($\mu\text{g/l}$) in 1982 ($R^2 = 0.1$, $p < 0.005$).

Table 3. Kikwawila village. Malaria parasitemia and low ($<100 \mu\text{g/l}$) serum retinol levels

Serum retinol level	1982		1983		1984	
	N	% parasitemic	N	% parasitemic	N	% parasitemic
$<100 \mu\text{g/l}$	9	89	17	59	3	100
$\geq 100 \mu\text{g/l}$	77	78	118	65	72	65

urinary schistosomiasis, giardiasis, necatoriasis and strongyloidiasis) (Tanner et al., 1987b). The few parasite free individuals (≤ 6) had the same mean retinol concentrations as had parasitized persons (190 vs 194 $\mu\text{g/l}$).

No relationship was found between the presence of single parasitic infections and average retinol levels, except for malaria parasitemia which was inversely correlated with retinol levels in the initial survey ($F = 9.6$, $p < 0.005$, Fig. 3). Pretreatment serum retinol concentrations in persons with or without giardiasis were comparable (Table 5).

The proportion of persons with retinol concentrations $<100 \mu\text{g/l}$ was not influenced by the presence of individual parasites (Table 3, showing malaria parasitemia).

For the subgroup changes of retinol status between 1982–1983 and 1983–1984 were compared with incidences of parasitic infections. In persons with increasing retinol levels, the risk of urinary schistosomiasis was 2.3 times lower than in persons with decreasing levels. In contrast, the risk of malaria parasitemia significantly increased with increasing retinol levels (Table 4).

Table 4. Kikwawila village. Change of retinol status and incidences of parasitic infections 1982–1983 and 1983–1984

	Retinol increase (N = 54)		Retinol decrease (N = 46)		Relative risk
	N	Incidence/1000	N	Incidence/1000	
<i>Relative risk <1</i>					
<i>S. haematobium</i>	24	167 ^a	21	381 ^a	0.4
Hookworm	19	316	10	400	0.8
<i>Relative risk >1</i>					
<i>S. stercoralis</i>	26	115	17	59	2.0
<i>G. lamblia</i>	48	146	30	67	2.2
<i>Plasmodium</i> spp.	17	765 ^b	10	400 ^b	1.9

^{a-a} $z_i = 1.62$, $p = 0.053$

^{b-b} $z_i = 1.89$, $p = 0.03$

Table 5. Kikwawila village. Treatment with ornidazole 40 mg/kg single dose, May 1983

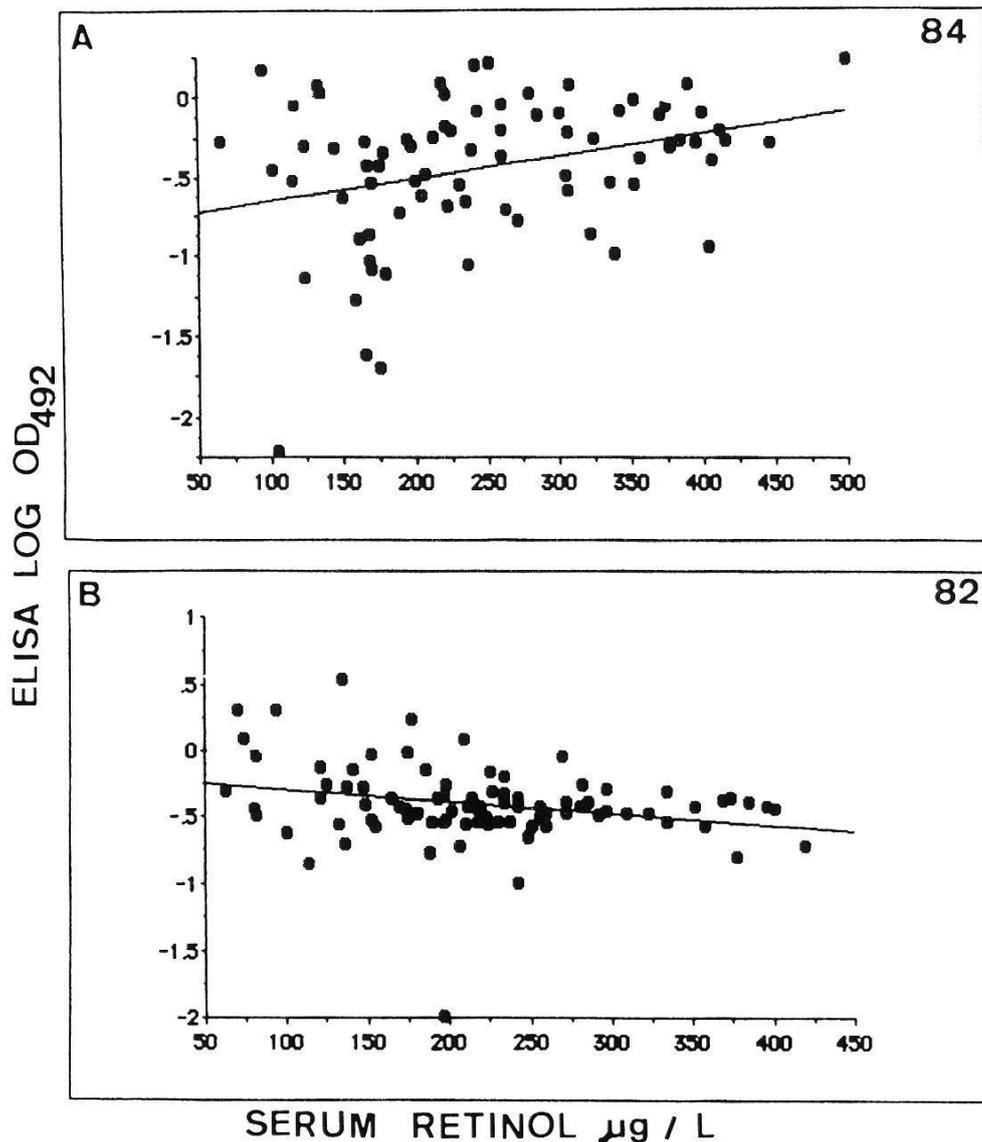
	<i>G. lamblia</i> present		<i>G. lamblia</i> absent	
	treated	not treated	treated	not treated
Number	11	22	48	52
Age (years): mean (\pm sd)	7.8 (3.4)	6.7 (4.3)	8.0 (3.8)	6.7 (3.9)
Sex ratio (males:females)	1.4	1.1	0.6	0.8
<i>Before treatment (October 1982)</i>				
Retinol (μ g/l): mean \pm sd	177 \pm 53	235 \pm 102	232 \pm 90	202 \pm 80
Midarm circumference (cm): mean ..	16.3	16.7	17.0	16.6
<i>P. falciparum</i> sporozoite ab titer ^a : gm mean	-0.49	-0.52	-0.63	-0.55
<i>Schistosoma</i> ab titer ^a : gm mean	-0.52	-0.58	-0.59	-0.57
<i>After treatment (October 1983)</i>				
Free of <i>G. lamblia</i> (%).....	90.9 ^b	63.6 ^b	-	-
Incidence of <i>G. lamblia</i> (%)	-	-	4.2	11.5
Retinol (μ g/l): mean \pm sd	210 \pm 97	177 \pm 55	190 \pm 84	197 \pm 95
Midarm circumference (cm): mean ..	17.3	17.5	17.6	17.2
<i>P. falciparum</i> sporozoite ab titer: gm mean	-0.30	-0.49	-0.56	-0.38
<i>Schistosoma</i> ab titer: gm mean	-0.49	-0.59	-0.67	-0.61

^a for description of test antigens see Materials and Methods

^{b-b} $z_i = 1.66$, $p = 0.049$

Ornidazole treatment. Untargeted mass treatment with a single oral dose of 40 mg/kg of ornidazole was performed in May of 1983. Six months after treatment 10 of 11 infected persons (91%) remained free of *G. lamblia* infection, compared to 64% in the control group ($z_i = 1.7$, $p < 0.05$, Table 5). Average retinol levels improved in the treated and infected group, but not in the three other groups (Table 5). Increments of height (data not shown), average MC measures and geometric mean antibody titers were not influenced by treatment.

Retinol and immune response. Antibody titers to *P. falciparum* sporozoites increased in parallel with increasing serum retinol concentrations. F values



A CSP PEPTIDE
B TETANUS TOXOID

Fig. 4. Correlation of antibody titers (log OD₄₉₂) in ELISA with retinol serum concentrations (µg/l). A: Antibodies to *P. falciparum* synthetic CS peptide, (NANP)₄₀ in 1984 ($y = 0.001x - 0.79$; $R^2 = 0.09$, $p < 0.01$). B: Antitetanus antibodies in 1982 ($y = 0.001x - 0.20$; $R^2 = 0.06$, $p < 0.005$).

were 3.3 (ns), 3.0 (ns) and 7.5 ($p < 0.01$) in 1982, 1983 and 1984, respectively (Fig. 4, showing results of 1984). However, the correlation was not maintained in persons > 5 years which could suggest confounding by age. No correlation was found between retinol concentrations and antibody titers to malaria blood stage antigen, or *S. mansoni* adult worm antigen. An inverse relationship was seen between retinol concentrations and antitetanus antibodies. F values were 5.8 ($p < 0.025$) and 3.1 (ns) in 1982 and 1984, respectively (no data were available for 1983, Fig. 4, showing results of 1982). Again, confounding by age could not be excluded.

Average serum retinol levels were comparable among seroreactors and nonreactors except in 1982 when 41 persons with *S. haematobium* antibodies had average retinol levels of 241 $\mu\text{g/l}$ compared to 198 $\mu\text{g/l}$ of 47 nonreactors ($t = 2.5$ $p < 0.01$), and 21 persons with elevated antitetanus antibodies had lower retinol levels than 67 nonresponders (162 vs 235 $\mu\text{g/l}$, ($t = 3.8$ $p < 0.0005$)). The proportion of persons with retinol levels < 100 $\mu\text{g/l}$ was equal in seroreactors and nonreactors (Table 6).

Table 6. Kikwawila village. Antibodies and low (< 100 $\mu\text{g/l}$) serum retinol concentrations

Antibody against	Test	Cutoff	1982		1983		1984	
			Retinol ($\mu\text{g/l}$)		Retinol ($\mu\text{g/l}$)		Retinol ($\mu\text{g/l}$)	
			< 100	≥ 100	< 100	≥ 100	< 100	≥ 100
<i>P. falciparum</i>								
(i) sporozoites	ELISA	≥ 0.3	3/6 (50) ^a	38/64 (59)	5/11 (46)	62/98 (63)	2/2 (100)	47/77 (61)
(ii) blood stage ags . . .	IFA	$\geq 1:80$	8/8 (100)	71/78 (91)	0/17 (0)	0/115 (0)	nd	nd
	ELISA	≥ 0.3	nd	nd	nd	nd	2/3 (67) ^b	16/87 (18) ^b
<i>S. mansoni</i>								
Adult worm ag	ELISA	≥ 0.25	4/8 (50)	37/79 (47)	9/17 (53) ^c	34/116 (29) ^c	0/3 (0)	30/92 (33)
Tetanus toxoid	ELISA	≥ 0.5	4/8 (50)	17/79 (22)	nd	nd	1/3 (33)	10/87 (22)

^a %

^{b-b} $\chi^2 = 4.2$, $p < 0.05$

^{c-c} $\chi^2 = 3.8$, $p \approx 0.05$

Discussion

One third of cross-sectionally examined persons could be followed for three consecutive years, and only about 30% of this cohort provided adequate amounts of sera for complete testing. Consequently, the sample was likely to be distorted, and the number of persons with complete data collection was small and often prohibited stratification for age.

Retinol deficiency is regarded a public health problem when >5% of children 6 months to 6 years of age present serum retinol levels <100 µg/l (WHO, 1982). This proportion was reached in 1982 and 1983, though few people had conjunctival xerosis, and none had severe forms of xerophthalmia. This discrepancy can be explained by the presence of latent retinol deficiency which has been observed elsewhere in Africa (Le François et al., 1980; Stürchler et al., 1983). In Mali for instance, 14% of 57 children 0 to 5 years old had retinol serum levels <100 µg/l without evidence of xerophthalmia (Le François et al., 1980). On the other hand, in the Ivory Coast of 430 children 7 to 9 years old 30% had retinol levels <200 µg/l, and another 30% had clinical signs of deficiency, mainly xerosis (Lauber and Haller, 1980). Alternatively, cases of conjunctival xerosis may have been overlooked in our study.

According to a food consumption survey, 92% and 7% of Kikwawila families consumed leafy vegetables during the lean (February) and post-harvest (August) seasons of 1983, respectively (Lukmanji and Tanner, 1985; Tanner and Lukmanji, 1987). This substantial seasonal dietary shift went in parallel with a decrease of average vitamin A intake (from 929 µg/day to 382 µg/day) and of the fraction of persons with adequate (FAO/WHO standards, Passmore et al., 1974) daily vitamin A intake (from 99% to 40%). These data are in agreement with our findings of low retinol levels towards the end of the dry season. The improved community retinol status observed in 1984 can be attributed to the entirety of health interventions (activities of village health workers, latrine campaign and mass treatment) during 1982 and 1983 (Tanner et al., 1987a, b).

Of all nutritional parameters, hematocrit and MC showed the closest correlation with age ($p < 0.0001$ for both parameters in all three years). Retinol (Tables 1 and 2, Fig. 1) and prealbumin levels were also correlated with age. Thus, age could confound the relations of retinol with MC (Fig. 2), prealbumin or hematocrit. Associations of serum retinol and retinol binding protein have been reported before (Arroyave, 1969; Atinmo et al., 1983; Stürchler et al., 1983); retinol binding protein binds to prealbumin in a 1:1 molar complex (Goodman, 1981). Retinol deficiency and anemia were linked together epidemiologically and biochemically (DeLuca et al., 1979). The association of retinol with MC was maintained in age groups 6–10 and 11–15 years, but not in age group 0–5 years. MC measurements appear to be stable between 1 and 5 years (King et al., 1978). The correlation of MC and retinol may be useful for nutri-

tional field surveys and primary health care programs for identification of populations at risk of retinol deficiency. Serum zinc levels were not correlated with age or vitamin A levels. Zinc supply is related to the zinc content of local vegetables which depends on the amount of zinc in farmland. Local variations of soil zinc levels were demonstrated in Kikwawila (Zehnder et al., 1986).

Ornidazole is effective against infections with *E. histolytica*, *G. lamblia* and anaerobic bacteria (Iyngkaran et al., 1978; Degrémont et al., 1981; Jokipii and Jokipii, 1982; McLean et al., 1984). Six months after administration of a single oral dose of 40 mg/kg a significant effect on the prevalence of *G. lamblia* in the community could still be observed (Table 5) (Tanner et al., 1987a, b). Only in persons with demonstrated *G. lamblia* infections and ornidazole treatment did average retinol levels increase from 1982 to 1983 (Table 5). The effects of light infections with *A. lumbricoides*, *G. lamblia* or *S. haematobium* on nutritional status and growth of preschool children are difficult to investigate in the field, and conflicting results have been reported (Gupta et al., 1977; Freij et al., 1979; Mahalanabis et al., 1979; Willett et al., 1979; Brown et al., 1980; Chavalittamrong et al., 1980; Stephenson et al., 1980; Greenberg et al., 1981; Gupta and Urrutia, 1982; Stürchler et al., 1983; Mandour et al., 1984). One problem is the proper parasitological diagnosis. *G. lamblia* trophozoites are not well preserved in MIF solution which was used in this survey. *G. lamblia* is shed irregularly, and one stool sample may detect <80% of infections. The density of *G. lamblia* in the stool does not correlate with clinical symptoms. Therefore it was not surprising that neither the demonstration of *G. lamblia* in the stool nor the demonstration of antibodies to *G. lamblia* in the serum was associated with low serum retinol levels.

Another difficulty is to identify single nutrient and parasite effects. A combined effect of giardiasis, malaria, necatoriasis, strongyloidiasis and schistosomiasis on annual weight or MC gains has been identified in the Kikwawila population following mass treatment (Tanner et al., 1987b). In a study of schoolchildren in northern Tanzania only the combined effects of ascariasis, ankylostomiasis and schistosomiasis had a significant effect on nutritional status as determined by weight-for-age <80% of standard (Meakins et al., 1981). Iron deficiency (Murray et al., 1975 and 1978a; Oppenheimer et al., 1984), regular milk diet (Murray et al., 1978b and 1980), and riboflavin deficiency (Thurnham et al., 1983) which are suspected to modify malaria morbidity can be interrelated.

A third difficulty is that exposure, parasite load and immune response to parasitic infections are age-related. Thus, we could not determine whether the inverse association of malaria parasitemia with retinol levels (Fig. 3) was confounded by age, or real. Likewise both possibilities would explain the association of antibody titers to *P. falciparum* sporozoites with retinol concentrations (Fig. 4). However, that increasing retinol levels increased the risk of malaria but at the same time reduced the risk of schistosomiasis (Table 4) suggests, that

retinol may contribute to regulate the load with selected parasites. This effect might be mediated by enhancing the stabilisation of membranes (De Luca et al., 1979) or by stimulating the cellular immune response (in particular T-cell activity) for which there is experimental and some clinical evidence (Anonymous, 1986).

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