

Toxic Damage to the Respiratory Epithelium Induces Acute Phase Changes in Vitamin A Metabolism without Depleting Retinol Stores of South African Children^{1,2,3}

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ABSTRACT Whereas there is much information concerning the effects of vitamin A status on response to infectious challenge, the effects of infection or trauma on vitamin A metabolism and status are less well documented. These relationships need to be understood to optimize clinical and public health programs to improve vitamin A status and health of children in less-developed countries. We measured acute changes in retinol and retinol-binding protein in 57 young South African children hospitalized following respiratory epithelial damage resulting from accidental ingestion of kerosene. In addition, vitamin A status, as measured by the modified relative dose response test, of these children 3 mo later was compared with that of neighborhood control children to determine whether their illness had depleted retinol stores. Plasma retinol was already significantly below control levels when children were admitted [geometric mean (95% CI): 0.57 $\mu\text{mol/L}$ (0.48–0.67) compared with 1.15 $\mu\text{mol/L}$ (1.02–1.30) for controls] and decreased further the following morning [0.38 $\mu\text{mol/L}$ (0.31–0.46)]. Significant differences in retinol-binding protein were not detected until the next morning [5.99 mg/L (4.70–7.63) compared with 14.0 mg/L (11.8–16.6) for controls] and were not as large as the relative differences in retinol. This dissociation between changes in retinol and its binding protein suggests that there may be increased retinol uptake by certain tissues during the acute phase response. The proportion of case children (37/46, 80%) with inadequate liver retinol stores 3 mo after the illness was slightly, but not significantly ($\chi^2 = 2.16$, $P = 0.14$), greater than the proportion of control children (28/42, 67%). Acute respiratory illness therefore did not further deplete retinol stores in this population in which stores were already frequently inadequate. *J. Nutr.* 127: 1339–1343, 1997.

KEY WORDS: • vitamin A • acute phase response • respiratory • kerosene • children

Vitamin A plays major roles in maintaining the integrity of epithelia (Wolbach and Howe 1925) and optimal function of the immune system (Ross 1992). These roles are presumed to be responsible for the major beneficial effects of improving vitamin A intake on mortality, particularly from diarrhea, of young children in less-developed countries (Beaton et al. 1993, Ghana VAST Study Team 1993). Although the respiratory epithelium is highly sensitive to vitamin A deficiency, vitamin A supplements have little effect on respiratory morbidity unless it is associated with measles (Vitamin A and Pneumonia Working Group 1995). Therefore, understanding the interactions among vitamin A, infection, epithelia and immunity has both clinical and public health importance.

That decreases in plasma retinol are characteristic of acute phase responses to infection or trauma has been known for decades (Ramsden et al. 1978, Tabone et al. 1992). Mechanisms that have been suggested are losses of holo-retinol binding protein (holo-RBP)⁵ in the urine (Alvarez et al. 1995, Donaldson et al. 1990, Ramsden et al. 1978, Stephensen et al. 1994), decreased release of RBP from the liver (Rosales et al. 1996, Thurnham and Singkamani 1991) and loss of holo-RBP into the extracellular fluid due to increased vascular permeability (Thurnham and Singkamani 1991). However, all of these mechanisms for lowering plasma retinol during acute phase responses postulate losses of retinol bound to RBP and ignore evidence that the molar decreases in plasma retinol during infection or trauma frequently seem to be greater than the decreases in plasma RBP (James et al. 1984, Ramsden et al. 1978, Reddy et al. 1986, Samba et al. 1990). This suggests that during the acute phase response there may be a specific

¹ Presented in part at the annual meeting of the British Nutrition Society, July 1996, Coleraine, Northern Ireland [Willumsen, J. F., Simmank, K., Filteau, S. M., Wagstaff, L. A. and Tomkins, A. M. (1996) Effect of acute toxic epithelial damage on vitamin A metabolism and status in South African children].

² This study was financially supported by the Wellcome Trust.

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⁵ Abbreviations used: AGP, alpha₁-acid glycoprotein; CRP, C-reactive protein; DR, diidehydroretinol; MRDR, modified relative dose response; RBP, retinol-binding protein; RDR, relative dose response.

uptake of retinol into tissues, presumably those tissues with a particularly high requirement to combat the traumatic or infectious stress. An important corollary to this hypothesis is that the lowered plasma retinol during the acute phase response does not necessarily signify a deficiency but may be a beneficial adaptation to the imposed stress.

Within a few weeks of resolution of a traumatic or infectious insult, plasma retinol increases to normal both in well-nourished adults in developed countries (Ramsden et al. 1978, Tabone et al. 1992) and in children from less-developed countries who have much lower retinol stores (Coutsoudis et al. 1991, Reddy et al. 1986), regardless of whether the children were given a large dose of vitamin A during the acute illness (Coutsoudis et al. 1991). However, these normal plasma retinol concentrations may mask a depletion in liver retinol stores (Campos et al. 1987).

We report here both the acute changes in vitamin A metabolism and the effects on vitamin A stores 3 mo later in South African children suffering from toxic damage to the respiratory epithelium as a result of accidental kerosene ingestion. Kerosene ingestion is, unfortunately, a common and serious cause of childhood poisoning in under-developed urban areas of South Africa and other poor countries (De Wet et al. 1994). The pathology appears to be primarily localized to the lung with other manifestations, for example, in the central nervous system, being secondary to hypoxia and acidosis (Klein and Simon 1986). With supportive treatment, most children recover from the acute effects of kerosene ingestion. Epithelial damage occurring in such circumstances, unlike infectious damage, lends itself to study of the time courses of acute changes in retinol metabolism. In black South African children, poor vitamin A status is common, with one third of children having serum retinol less than $0.7 \mu\text{mol/L}$ (South African Vitamin A Consultative Group 1996), so any major effects on vitamin stores are likely to be observable. Therefore, this study was conducted to determine whether the acute and chronic changes in vitamin A metabolism and status support the use of vitamin A supplements in the treatment of the injury.

MATERIALS AND METHODS

Patients. The study was conducted at Baragwanath Hospital, Soweto, South Africa. Children, aged 1–5 y, presenting at the hospital suffering pneumonitis as a result of accidental kerosene ingestion were eligible for the study. Additional inclusion criteria were previous good health as reported by the parent, residence within Soweto, and written informed consent from the parent or guardian. The study was approved by the ethical review committees of the University of Witwatersrand, Johannesburg, and the Institute of Child Health, London.

Of 275 children seen at Baragwanath Hospital for kerosene ingestion during the study period (December 1994 to January 1996), there were two deaths, both within 24 h, and 57 children, including the most severe cases plus a convenience sample of milder cases, were recruited for the study. Details of the clinical findings and morbidity of the children during a subsequent 3-mo follow-up will be published separately. Briefly, on admission children were examined, a history was taken that included the time when kerosene was ingested, and blood samples were taken. Children were observed overnight and standard supportive treatment, usually anti-pyretics and frequently oxygen, was given as required and according to the hospital protocol. Antibiotics were not routinely given, and there was no evidence that infection was a problem in any patients. The following morning, children were examined again, a further blood sample and a spot urine sample were collected, and children were either discharged ($n = 30$) if respiratory symptoms, but not necessarily fever, had resolved, or admitted ($n = 27$) if oxygen requirements persisted (maximum 18 d).

Children were followed up in the community every 2 wk for the following 3 mo to monitor morbidity. At the first visit, an apparently

healthy neighborhood control child of similar age was also recruited. Three months after kerosene ingestion, both case and control children were brought to hospital for examination by one of the study physicians. At this time a modified relative dose response (MRDR) test for liver vitamin A stores (Tanumihardjo et al. 1996) was conducted using dihydroretinol (DR) obtained from S. Tanumihardjo (Iowa State University). Seven of the 57 cases were not found at the address given in hospital so sample size for follow-up data was reduced to 50, all but three of whom completed the 3-mo follow-up and gave final blood samples. Of 50 controls recruited, eight dropped out before 3 mo but three additional children were recruited for the final visit only, giving 45 control blood samples. Data for age were missing from seven control children.

Laboratory analyses. Urine samples were treated soon after collection with about 0.5 g of sodium bicarbonate to stabilize RBP (Donaldson et al. 1990) and then frozen. Blood samples were centrifuged and serum collected within several hours in the hospital laboratory. Serum was then frozen at -70°C until shipped on dry ice to London for analysis. Serum retinol was analyzed by HPLC (Filteau et al. 1993). A similar protocol was followed for measurement of serum DR as part of the MRDR tests, the only differences being the use of $200 \mu\text{L}$, rather than $100 \mu\text{L}$, of sample, and measurement at 350 nm, rather than 325 nm; both modifications were to increase sensitivity of the DR measurement. For a quality control included in duplicate in each of six analyses, the intra- and interassay coefficients of variation were 2.7 and 5.5% for DR, respectively, and 2.6 and 6.4% for retinol. Two acute phase proteins, C-reactive protein (CRP) and alpha₂-acid glycoprotein (AGP) were measured by ELISA (Filteau et al. 1994, Gillespie et al. 1991) to monitor the acute phase response to the initial injury and any infection at the time of the 3-mo follow-up blood sampling. Retinol-binding protein was measured in both blood and urine by a sandwich ELISA using rabbit antibodies to human RBP (Dako, High Wycombe, UK), without and with horseradish peroxidase, as the capture and conjugated antibodies, respectively. The RBP standard was from Behring (Hoechst Roussel, Milton Keynes, UK) and the interassay coefficient of variation for a quality control serum analyzed in duplicate on each plate was 11.3% ($n = 43$). Urine RBP levels were compared with creatinine measured by the Jaffe method. A urine RBP/creatinine ratio of $>42 \mu\text{g}/\text{mmol}$ is used to diagnose renal failure in children (V. Shah, Great Ormond Street Hospital, London, UK, personal communication).

Statistical analyses. Data were entered into EPI-Info (CDC, Atlanta, GA and WHO, Geneva, Switzerland), which was also used for descriptive statistics. The case group at each time point was compared with the healthy controls at 3 mo. Detailed analyses were conducted in SPSS/PC (SPSS Inc., Chicago, IL) according to the procedures of Kirkwood (1988). All biochemical data except hemoglobin and AGP concentrations were log-transformed to normalize distributions. Data were analyzed by analysis of variance with potential confounding factors, e.g., age, sex, and severity of the acute illness, defined as days of hospital stay, included as covariates. The chi-square test was used to compare proportions. Significance was at the 5% level.

The sample size of 50 was chosen as sufficient to detect an increase from 10% to 40% in the percentage of children with abnormal MRDR results at 3 mo after kerosene ingestion. However, this turned out to be an underestimate of the prevalence of low liver vitamin A stores in this population so that, with the true prevalence of abnormal MRDR results in control children of 67%, the sample size was sufficient to detect a difference of 25% (i.e., 92% prevalence of abnormal results in case children) at 5% significance and 80% power.

RESULTS

Table 1 compares characteristics of case and control children at the final follow-up. There were no significant differences between the two groups. As is typical for accidental injury, there were more boys than girls. Although the cases and controls could not always be matched for sex, there are no reported sex differences in key outcomes such as serum retinol in South African children (South African Vitamin A Consultative Group 1996) or MRDR results in Indonesian children (Tanumihardjo et al. 1996). The population was,

TABLE 1

Description of case and control children who completed follow-up¹

	Cases	Controls
Age, months	23.4 (10.1)	26.1 (9.6)
Sex [n (%)] F	11 (23%)	18 (40%)
Weight/age (Z)	-0.06 (1.57)	-0.27 (0.84)
[n (%) Z < -2]	5 (11%)	0 (0%)
Height/age (Z)	0.20 (1.50)	0.35 (1.09)
[n (%) Z < -2]	2 (4.3%)	2 (5.3%)
Weight/height (Z)	-0.15 (1.16)	0.08 (0.85)
[n (%) Z < -2]	4 (8.5%)	0 (0%)
Hemoglobin g/L	110 (11)	112 (11)
[n (%) <110]	20 (47%)	17 (44%)

¹ Values for continuous variables are means (SD) and are from measurements taken at the final follow-up visit. There were 47 cases and 45 controls, but age was missing for seven controls, thus reducing sample size to 38 where this is relevant. Hemoglobin values were available for only 42 cases and 39 controls. There were no significant differences between case and control children.

overall, slightly underweight, but severe undernutrition (Z scores < -2) was rare. Mild anemia (hemoglobin < 110 g/L) was common, but only two children, both cases, had hemoglobin < 90 g/L.

Figure 1 illustrates acute phase protein levels at admission, the following morning and 3 mo later for cases and at the 3-mo time point for control children. At admission, a mean of 6 ± 6.7 h (mean $\pm$ SD) after reported time of kerosene ingestion, CRP levels were already significantly greater than control levels and were elevated further the following morning. The AGP levels did not differ significantly from control levels at either sampling time during hospitalization. It is unlikely that peak levels of these proteins had been reached within the time samples could be taken (Curtis et al. 1995). These results

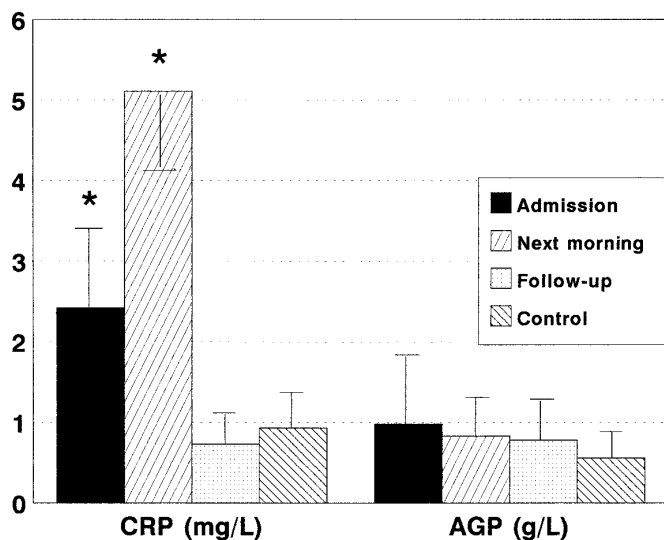


FIGURE 1 Serum acute phase proteins during hospitalization of case children who ingested kerosene and of case and control children 3 mo later. Values for C-reactive protein (CRP) are geometric means $\pm$ 95% confidence interval. Values for alpha₁-acid glycoprotein (AGP) are means $\pm$ SD. Sample size was 54 at admission, 43–45 for cases or controls at other time points. *Values for CRP at admission and the next morning were significantly greater than controls at 3 mo ($P < 0.05$). Values for AGP at all time points were not significantly different.

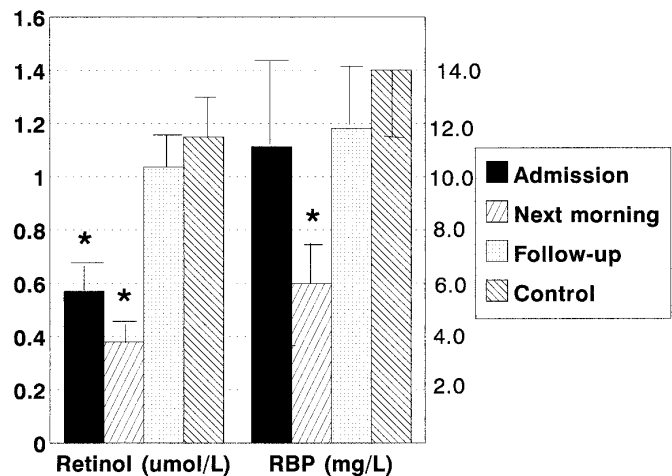


FIGURE 2 Serum retinol and retinol-binding protein (RBP) during hospitalization of case children who ingested kerosene and of case and control children 3 mo later. Values are geometric means $\pm$ 95% confidence intervals. Sample sizes were 53 (retinol) or 54 (RBP) at admission and 42–46 for cases or controls at other time points. *Values for retinol at admission and the next morning and values for RBP the next morning were significantly different from corresponding control values at 3 mo ($P < 0.05$).

indicate that kerosene ingestion induces acute phase protein changes that follow a time course and, within 24 h, are of a magnitude typical of mild systemic injury or infection (Thompson et al. 1992). At 3 mo later, CRP and AGP levels were not different in case and control children. However, a significantly ($\chi^2 = 5.59$, $P < 0.05$) higher proportion of the cases (13/47) than controls (1/45) had AGP levels at 3 mo greater than 0.75 g/L, the upper limit of normal for this protein in British adults measured by our method (Filteau et al. 1994). Although at this time there was a greater prevalence of cough in case (55%) compared with control (29%) children, raised AGP levels were not significantly associated with any observed morbidity symptoms.

At time of admission (Fig. 2), plasma retinol of case children was already significantly below control levels [geometric mean (95% CI): 0.57 μ mol/L (0.48–0.67) for cases, 1.15 μ mol/L (1.02–1.30) for controls] and decreased further the following morning [0.38 μ mol/L (0.31–0.46)]. At admission, however, serum RBP of cases [11.1 mg/L (8.7–14.3)] did not differ from values for controls [14.0 mg/L (11.8–16.6)], although it was significantly less by the next morning [5.99 mg/L (4.70–7.63)]. The decreases in retinol thus seemed to be both faster and more profound than the decreases in RBP. At the 3-mo follow-up, there was no difference between case and control children in either retinol or RBP level. Retinol levels at this time were characteristic of moderate subclinical vitamin A deficiency in the population (WHO/UNICEF 1992), with 8/46 (12%) case children and 5/42 (12%) control children having serum retinol less than 0.7 μ mol/L.

Urine RBP/creatinine ratio was significantly elevated in case children during the acute phase (mean: 37.4 μ g/mmol, 95% CI: 23.5–59.6, $n = 46$) compared with values in case (8.5 μ g/mmol, 5.2–13.8, $n = 41$) and control (10.1 μ g/mmol, 7.1–14.5, $n = 43$) children at 3 mo. During the acute phase, 18/46 (39%) cases had a raised RBP/creatinine ratio (>42 μ g/mmol), compared with 4/41 (10%) case and 3/43 (7%) children at the 3-mo follow-up. Urinary RBP loss was significantly correlated with both serum retinol ($r = -0.35$, $P = 0.018$, $n = 45$) and serum RBP ($r = -0.36$, $P = 0.015$, $n = 46$) at

admission but not significantly the next morning (data not shown).

Liver retinol stores, as reflected by MRDR results, were inadequate, i.e., MRDR greater than 0.06 (Tanumihardjo et al. 1996) in a large proportion of both case (37/46, 80%) and control (28/42, 67%) children at the 3-mo follow-up point. These proportions did not differ significantly ($\chi^2 = 2.16$, $P = 0.14$). There was no significant effect of the severity of the acute illness in the case children, defined dichotomously as whether children were discharged after an overnight stay or stayed longer, on the proportion abnormal MRDR (18/23 abnormal in the less ill, 18/22 abnormal in the more severely ill). Although case children had slightly more fever and diarrhea than controls and similar amounts of respiratory symptoms during the 3-mo follow-up, this subsequent illness in both case and control children, defined as the sum of days with diarrhea and days with cough, was not significantly correlated with MRDR ($r = -0.11$, $P = 0.33$, $n = 79$).

DISCUSSION

Kerosene ingestion provided as close as ethically possible a model system for a controlled study of inflammation-induced changes in vitamin A metabolism and status in children of marginal vitamin A status. Unlike the more common situation of infection-induced acute phase responses in children in less-developed countries, in which the time of initial infectious challenge is generally unknown, kerosene poisoning allowed us to document the time course of the acute phase changes. Also unlike the case of infection, kerosene ingestion is presumably independent of prior vitamin A status, and there is no evidence that vitamin A status affects death from accidental injury (Ghana VAST Study Team 1993). We minimized the possible confounding factor of low socioeconomic status, which is associated with both vitamin A deficiency and accidental injury, by comparing the status of case children at follow-up with that of neighborhood controls. Therefore, we believe that our results can be extrapolated to effects of common childhood infections on vitamin A metabolism and status.

The model system allowed us to improve understanding of both acute and more chronic changes in vitamin A metabolism following an acute inflammatory stress. The decreases in plasma retinol that occurred as a result of the acute inflammation were more rapid and of relatively greater magnitude than the decreases in plasma RBP. We are unaware of previous data showing such dissociation in the time courses for these changes, presumably because others have not examined such rapid changes (Ramsden et al. 1978). However, this decreased saturation of plasma RBP is a common feature of infection in both animals (Sijtsma et al. 1989) and humans (James et al. 1984, Ramsden et al. 1978, Reddy et al. 1986, Samba et al. 1990). Therefore, our results suggest that mechanisms in addition to decreased secretion of holo-RBP from the liver or increased excretion of this complex into the urine (although this was considerable and negatively associated at one time point with serum retinol) or leakage into extravascular spaces are responsible for the decreased plasma retinol characteristic of acute phase responses. Possible mechanisms include increased degradation or uptake of retinol into tissues, such as epithelia or immune cells, which may particularly require it to combat the illness. These mechanisms could best be studied using radioisotopes in experimental animals.

Increased tissue uptake, particularly if accompanied by irreversible retinol catabolism, could potentially deplete liver retinol stores. However, rodents can decrease the rate of degradative utilization of vitamin A under conditions of vitamin A

deficiency (Green and Green 1994), and hence it is possible that similar mechanisms could limit retinol depletion in individuals of poor vitamin A status who suffer infection or trauma. There is some information on tissue concentrations after infection or acute inflammation in experimental animals, but this is inconsistent and suggests that changes in retinol distribution depend on the nature of the inflammatory (Kanda et al. 1990, Rosales et al. 1996) or infectious (Sijtsma et al. 1989, Wang et al. 1994, West et al. 1992) stress.

Most data concerning the effects of infection on vitamin A stores of humans are circumstantial, with few direct measurements of these stores. The results are generally confounded by the well-established reverse relationship, i.e., the effect of poor vitamin A status on increasing severity of infection. Therefore, although diarrhea or respiratory disease was associated with subsequent development of xerophthalmia in children (Sommer et al. 1987) or with poor vitamin A stores in spite of supplementation (Rahman et al. 1996), it is possible that the children who became ill were already the most vitamin A deficient. The most conclusive study, from Brazil, showed an increase in abnormal RDR results, i.e., decreased vitamin A status, in children after chicken pox infection (Campos et al. 1987). This study was actually designed to test the length of time a 60-mg dose of retinol could maintain adequate liver stores in children, and so all children had adequate vitamin A status, as assessed by RDR, before the infection.

Our present study overcomes the problem of bidirectional interactions between vitamin A and infection by investigating effects on vitamin A stores of an accidental injury, susceptibility to which was unlikely to have been influenced by prior vitamin A status. Kerosene ingestion resulted in no significant depletion of liver retinol stores. The lack of difference between cases and controls, however, may have been partly a result of the sample size, which turned out to have inadequate power given the much higher prevalence than expected of low liver retinol levels in the control children. Nevertheless, our results are supported by a small study showing no difference in the proportion of abnormal MRDR of 23 young Nigerian children 5 wk after hospitalization for pneumonia compared with 23 neighborhood controls (Sotimehin, A., Naik, R., Willumsen, J. and Filteau, S., unpublished results).

The possible differences between our study, showing no depletion of vitamin A stores, and the very similar study on chicken pox infection, in which vitamin A stores were depleted (Campos et al. 1987), are of interest. Both kerosene ingestion and chicken pox are illnesses of moderate severity from which children usually recover virtually completely, although this may take longer after chicken pox than after kerosene ingestion. The major pathology in chicken pox is systemic, whereas kerosene poisoning, although it does induce fever and an acute phase response, has effects localized primarily to the respiratory tract. It would be of interest to study the effects of other illnesses affecting other sites, for example, diarrhea, on vitamin A stores. Another possible difference between the studies is that the children in Brazil all had been recently dosed with vitamin A and had adequate stores before their illness, whereas in both the present study and our small one in Nigeria, most children had inadequate stores. If children can adapt and conserve vitamin A when stores become depleted, as occurs in rats, their vitamin A metabolism may have been differently affected by a superimposed insult.

The high prevalence of abnormal MRDR results was a surprise, even given the greater sensitivity of MRDR than serum retinol for detecting vitamin A deficiency, considering that dietary vitamin A sources were not uncommon (unpublished observations) and that serum retinol data indicated vitamin

A deficiency was a moderate subclinical public health problem in this population (WHO/UNICEF 1992). We found a similar discrepancy in our results from Nigerian children, and these were supported by similar prevalences of both low serum retinol and abnormal MRDR in children from the same area in a 1994 Nigerian national survey (Atinmo, T., University of Ibadan, Ibadan, Nigeria, personal communication). The proportion of abnormal MRDR results in our study was intermediate between the proportions of children with deficient liver retinol stores in Bangladesh (Rahman et al. 1996) and Indonesia (Tanumihardjo et al. 1996).

Because supportive therapy is generally sufficient for recovery of children who ingest kerosene and because there seems to be little excess subsequent morbidity (Simbank, K., Wagstaff, L., Sullivan, K., Filteau, S. and Tomkins, A., unpublished results), large doses of vitamin A given to children when admitted to the hospital with respiratory illness resulting from kerosene ingestion are unlikely to improve recovery from the acute injury. However, particularly in such areas as South Africa, where vitamin A deficiency is known to be prevalent, it would be appropriate to take this opportunity to give supplements in order to improve vitamin A status. Discharge from hospital might be a more effective time for supplementation to avoid possible malabsorption or urinary loss during the acute illness.

ACKNOWLEDGMENTS

We wish to thank Chrissie Mkhasibe for dedicated follow-up of the children, Thoreso Phutheho for driving children for follow-up visits, John Raynes, London School of Hygiene and Tropical Medicine, for use of his laboratory space and HPLC expertise, and Rina Naik for conducting HPLC assays.

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