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PATHOGENESIS OF CANCRUM ORIS (NOMA): CONFOUNDING INTERACTIONS OF MALNUTRITION WITH INFECTION

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Abstract. This study showed that impoverished Nigerian children at risk for cancrum oris (noma) had significantly reduced plasma concentrations of zinc (< 10.8 μmol/L), retinol (< 1.05 μmol/L), ascorbate (< 11 μmol/L), and the essential amino acids, with prominently increased plasma and saliva levels of free cortisol, compared with their healthy counterparts. The nutrient deficiencies, in concert with previously reported widespread viral infections (measles, herpesviruses) in the children, would impair oral mucosal immunity. We postulate, subject to additional studies, that evolution of the oral mucosal ulcers including acute necrotizing gingivitis to noma is triggered by a consortium of microorganisms of which *Fusobacterium necrophorum* is a key component. *Fusobacterium necrophorum* elaborates several dermonecrotic toxic metabolites and is acquired by the impoverished children via fecal contamination resulting from shared residential facilities with animals and very poor environmental sanitation.

Noma, also known as cancrum oris, is an infectious disease that destroys the oro-facial tissues and neighboring structures in its fulminating course, and has a mortality rate as high as 70–90% if not promptly treated.[1–4] This disease was known to Hippocrates, Galen, Celsus, and Aretaeus of Cappadocia, and was also widely reported in European and North American writings of the 18th and 19th centuries.[1] Noma virtually disappeared from the affluent developed countries in the 20th century except for several cases found in the concentration camps of Belsen and Auschwitz,[5] and more recently, in association with intense immunosuppressive therapy,[6] in patients with human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome(AIDS),[7] as well as in Native American children with underlying severe combined immunodeficiency syndrome.[8] In marked contrast, noma is an escalating public health scourge of impoverished children in the developing world, particularly in sub-Saharan Africa where the frequency in several countries is estimated to be 1–7 cases per 1,000 population, and as many as 12 cases per 1,000 in the most affected communities.[3,4]

Although noma is observed in adults,[9] the disease typically affects mostly children between three and 12 years of age.[1,10,11] Poverty is the most important cause of noma in Africa,[3] and chronic malnutrition is a major predisposing factor.[1,2,12,13] A background of poor oral and general hygiene characterizes children at risk for this disease, and most cases of noma present with a distinct history of recent prior debilitating infections, with measles being the most frequent,[1,2,14] affecting 30 (43%) of 69 Nigerian children who subsequently developed noma.[2] Other antecedent infections reported in these 69 noma cases by Enwonwu[2] included malaria (30%), chicken pox (9%), tuberculosis and others (17%). It must be underscored that in impoverished African children, single infections are very rarely encountered. Measles infection is known to down-regulate interleukin-12 (IL-12), which is needed to generate cell-mediated immunity,[15] and studies have shown that malnourished children with compromised vitamin A status are prone to develop deep erosive ulcers of the mouth and eyes following measles attack.[16,17]

The popular belief is that noma is preceded by acute necrotizing gingivitis (ANG),[2,13] a severe, often painful form of gingivitis characterized by a necrotizing inflammation of the marginal interdental gingiva with little or no osseous involvement.[18] In sub-Saharan Africa, ANG is seen almost exclusively in economically underprivileged children usually 2–7 years of age.[2,19] The etiology of ANG is poorly defined and the reported risk factors include stress, poor oral hygiene, and immune suppression resulting from malnutrition as well as from bacterial and viral infections.[2,11,20,21] Our recent studies suggest that infections by the human herpesviruses, particularly the human cytomegalovirus (HCMV), contribute to the onset and progression of ANG in impoverished Nigerian children.[22] Nonetheless, only a relatively small percentage of ANG cases appear to evolve into noma.[2,23] The very rapid speed with which the orofacial lesion of noma establishes itself and the associated prominent foul odor, suggest that a collagenase type of enzyme, usually elaborated by anaerobes, is involved in the disease process.

We have hypothesized that the factors that distinguish underprivileged African children who develop noma from those who do not are an overlay of malnutrition and poor hygiene–borne microorganisms, particularly some specific Gram-negative anaerobes, as well as other factors (e.g., measles and other viral infections) that promote immune suppression and cause oral mucosal ulcers in the host.[3] Additionally, we have speculated that the environmental basis for high frequency of noma in some impoverished African communities is related to poor sanitation and possible contamination from livestock.[3,4] The latter suspicion derives from the observation of very close residential proximity of vulnerable Nigerian children to livestock,[3] as well as published reports that gangrenous processes similar to noma in the human, occur in the latter, and attributed to infection by *Fusobacterium necrophorum.*[24–26] Significantly more anaerobes, particularly *Prevotella intermedia,* are present in the mouths of malnourished Nigerian children compared with their well nourished counterparts, regardless of the presence or absence of any overt oral pathology.[27] Ongoing studies in Nigerian children indicate recovery of *F. necrophorum* from 88% of

the noma lesions.[28] *Prevotella intermedia,* alpha-hemolytic streptococci, and *Actinomyces spp.* were isolated from 75%, 50%, and 38% of the patients, respectively. This paper presents the pertinent nutritional/biochemical findings in Nigerian children with and without noma, and examines the pathogenesis of this disease in the light of these findings, their pathophysiologic implications, and our previously reported microbiologic data.

PATIENTS AND METHODS

This study was carried out with the prior approval of the Institutional Review Board, University of Maryland School of Medicine and the Ministries of Health and Education in the relevant states in Nigeria. Informed consent was obtained from the children's parents or legal guardians, as well as from the village chiefs where applicable.

Subjects. During the period June 1996 to October 1997, more than 1,000 Nigerian children, drawn from various socioeconomic strata, but mainly from the economically deprived rural communities and urban ghettos, were examined by a combined team of investigators from the University of Maryland (Baltimore), the Nigerian Institute for Medical Research (Lagos), and the University of Lagos School of Dental Sciences (Idi-Araba, Lagos). The subjects were approximately 2–16 years of age, and the study locations included preschool centers, primary schools, primary health care centers, and occasionally, out-patient clinics of tertiary hospitals in various states and local government areas in the country. The sampling strategy used required that we identify nutritionally deprived communities where, according to all available information, the prevalence of noma was reported to be high. Preliminary studies by our Nigerian colleagues and a Dutch-German medical team working in Nigeria had earlier identified Sokoto State as one of the Nigerian states with unusually high frequencies of reported noma cases.[29] Therefore, we established a temporary center at a Sokoto Hospital, to which fresh noma cases from the surrounding impoverished communities were brought in by primary health care nurses familiar with the communities. Every effort was made to match impoverished children (with and without ANG) from the same communities with each of the noma cases identified. Because of severe logistic constraints, our study was essentially combined cross-sectional and case control in nature. Following biological sampling of the cases, our Nigerian collaborators paid subsequent visits to the center and most of the noma cases were referred to a Dutch-German medical team for necessary surgical treatment. In addition to the impoverished children (with and without noma or ANG), we also collected biological samples from well-nourished, healthy, age-matched children from educated homes.

Sokoto State, located in the northwest corner of Nigeria, is bordered on the north by Niger, on the west by Kebbi State, and on the east by Zamfara and Katsina States. The estimated population of the state in 1997 was 2.7 million, with more than 75% of the total population living in the rural areas. Approximately 75% of the people in these rural communities were illiterate or semi-literate. The predominant occupation was agriculture. A significant number of the children in the rural communities was never vaccinated against measles, whooping cough, and other common childhood diseases. The principal health problems of the people were mainly infections such as malaria, tuberculosis, measles, pneumonia, and diarrhea, among others.[29] All of the people in impoverished Sokoto rural communities resided in very poorly ventilated mud huts with thatched bamboo roofs and dirt floors. They often shared the unhygienic living facilities in very close proximity with their domestic animals, e.g., sheep, goats, rams, donkeys, cows, and fowl. These communities had no access to a reliable supply of safe drinking water, and usually obtained water from contaminated, shallow wells. Facilities for safe disposal of human and animal fecal wastes were grossly inadequate. For oral hygiene care, the people relied almost exclusively on chewing sticks.[30] Quite often, the mothers cleaned the children's mouths solely with fingers and water. Children in Sokoto rural communities were usually breast fed up to two years of age when they were weaned to monotonous, energy-poor, millet and corn-based diets.

Preliminary screening of study subjects. The following information was obtained from each child: name, gender, residential location, parents occupation, number of siblings, and other pertinent demographic data, particularly residential proximity to livestock, e.g., goats, rams, cattle, etc. Detailed information was obtained on each child's medical history, particularly any recent history of eruptive fevers, diarrhea, and malaria. With the collaboration of the Nigerian Institute for Medical Research and the National AIDS Task Force, the children were subsequently screened serologically for HIV-1 and HIV-2 infection.

Nutritional evaluation. In most of the homes, meal frequency, and time of eating were not rigidly set. Many family members, particularly children, usually ate directly from shared bowls, thus making it virtually impossible to accurately record each individual child's food intake. Additionally, it was not possible to use methods that required the parents/guardians to record the children's food consumption because of the high level of illiteracy in the communities. Nutritional evaluation of the children in this study relied mainly on anthropometric and biochemical data.

Anthropometry. Body weight was measured to the nearest 50 g using scales whose precision was frequently checked, particularly at the start of each session. Height was measured to the nearest millimeter. Assessment of nutritional status was based on weight-for-height (wasting) as an indicator of current state of nutrition, and on height for-age (stunting) as an index of past nutrition.[31] Using computer programs developed by the World Health Organization (Geneva, Switzerland) and the Centers for Disease Control and Prevention (Atlanta, GA) for nutritional anthropometry (Epi-Info version 5.0 lb, 1993), weight-for-age, height-for-age, and weight-for-height Z scores were calculated, and interpreted relative to the standard values of the National Center for Health Statistics published by the U.S. Department of Health, Education and Welfare.[32] The Z-score cut-off point chosen was -2 SD of the reference median.

Oral health status. Determination of the oral health status of each child was carried out by one of us (OS), using a standardized oral diagnosis form. Both gingival and plaque indices were assessed according to Loe and Silness.[33,34] A crater-like lesion involving the interproximal gingival papillae, usually covered by a pseudo-membranous slough, and

often clearly demarcated from the rest of the gingival mucosa by a linear erythema, was diagnosed as ANG.[35,36] Our operational definition of early noma was an ulcer exposing the underlying alveolar bone, with the ulceration quite often extending into the labiogingival fold and involving the mucosal surface of the cheek and/or lips.[1] Advanced noma with perforation through the face and/or palate presented no diagnostic problem. Sampling of the oral tissues and lesions for microbiologic studies, as well as the methods used to identify the specific viruses and bacteria, have been reported in previous publications.[22,28]

Biochemical studies. *Sample collection.* Venous blood was collected into heparinized tubes from each subject from 9:00 AM to 11:00 AM following an overnight fast or fasting for at least 4–5 hr. Care was taken to protect the blood samples from undue exposure to light, heat, and air. Under field conditions, the blood-filled vacutainer tubes were retained in an ice-cooled, opaque container until centrifuged (2,000 × *g* for 10 min) usually within 1 hr after collection, to separate the plasma that was divided into aliquots for storage at −70°C. The aliquot for a subsequent ascorbate assay was stabilized with an equal volume of freshly prepared 10% metaphosphoric acid before storage.[37] The aliquot for retinol assay was stored with minimal exposure to air and light, and this was best achieved by flushing the head space of the storage tube with nitrogen, and wrapping the tube in aluminum foil. The aliquot for a cortisol assay was stored in a siliconized tube. The plasma aliquot for a zinc assay was stored frozen in zinc-free plastic tubes.

Unstimulated whole saliva was collected using an orapette, an oral fluid-collecting and dispensing device (Trinity Biotech, Plc., Dublin, Ireland). Each child was asked to place a rayon ball in the mouth and move it around with the tongue for about 3 min until it was completely saturated with saliva. The rayon ball was then put back into the bottom receiver part of the device, and the saliva squeezed out into a collecting plastic tube using the plunger portion of the orapette to compress the rayon ball. Following centrifugation, a cocktail of protease inhibitors (0.05 M 6-amino-hexanoic acid, 0.005 M benzamidine hydrochloride, 0.001 M phenylmethylsulfonyl fluoride, in 0.02 M phosphate-buffered saline [PBS], pH 7.4) (final concentration 0.01%) was added to the saliva before storage at −70°C.

Ascorbate assay. Plasma concentration of total ascorbic acid (dihydro plus dehydro forms) was determined following derivatization with 2,4-dinitrophenylhydrazine. To form the bis-2,4-dinitrophenyl-hydrazone, 0.1 ml of protein-free plasma supernatant was mixed with 0.02 ml of a solution containing 3.0 g of 2,4-dinitrophenylhydrazine, 0.4 g of thiourea, 0.05 g of $CuSO_4 \cdot 5H_2O$ made up to a total volume of 100 ml with 9 N H_2SO_4 and incubated at 37°C for 3 hr. Following addition of 0.15 ml of ice-cold 65% H_2SO_4 and thorough mixing, the sample was kept for an additional 30 min at room temperature before the absorbance at 520 nm was determined in a Beckman Spectrophotometer DU-50 (Beckman Instruments, Palo Alto, CA) and compared with standard readings. For interpretation of the data, we considered plasma ascorbic acid values <11 μmol/L to signify frank deficiency, values between 11 and 23 μmol/L as a marginal or moderate risk of developing clinical deficiency signs, and values > 23 μmol/L as normal.[38]

Retinol assay. The measurement of plasma concentration of retinol was done according to the method described by Conley and others,[39] using the Beckman high-performance liquid chromatography (HPLC; Beckman System Gold, 166 Detector, Beckman Instruments). Plasma proteins were precipitated by the addition of a mixture of 350 :l of 1-butanol:acetonitrile (1:1) and 300 :l of potassium phosphate to 500 :l of sample. The analytical column used was a Bio-Sil C18 (250 × 4.6 mm, particle size = 5 μm) 90-5S column obtained from Bio-Rad Laboratories (Hercules, CA). Mobile phase A was a mixture of 0.02 mM ammonium acetate and acetonitrile (50:50). Mobile phase B was 0.2 mM ammonium acetate and acetonitrile mixture (5:95). All solvents used were of analytical grade. The flow rate was 1.5 ml/min. Ultraviolet absorption was read at 325 nm. All-trans-retinol (Sigma, St. Louis, MO) served as the standard. For the interpretation of vitamin A status, plasma concentrations less than 0.35 umol/L were considered deficient, and levels ranging from 0.35 to 1.05 umol/L were classified as low to marginal.[40–42]

Other biochemical assays. Plasma zinc concentration was determined using a Perkin-Elmer (Norwalk, CT) 1100B atomic absorption spectrophotometer.[43,44] Aqueous zinc calibrator prepared in diluted glycerol (5 ml/dL) served as the standard. In each analytical batch, quality control was verified by inclusion of aliquots of a commercially available serum control with predetermined zinc values. Plasma zinc levels less than 10.8 μmol/L were considered indicative of cellular depletion.[45]

Determination of cortisol levels in plasma and whole saliva was carried out with the Gamma Coat ^{125}I-Radioimmunoassay Kit (Incstar Corporation, Stillwater, MN). The kit contained test tubes coated with rabbit anti-cortisol serum, ^{125}I-labeled cortisol in PBS, 8-anilino-1-napthalene sulfonic acid with 0.02 M sodium azide preservative, cortisol-free processed human serum as the blank, PBS, and cortisol standards in processed human serum. The radioimmunoassay kit, although designed for serum, plasma, or urinary cortisol determination, was easily adapted for saliva cortisol measurement.[46] The reference ranges for plasma cortisol levels were 193–690 nmol/L (mornings) and 55–248 nmol/L (evenings).

Levels of free amino acids in plasma were measured by reverse-phase HPLC following precolumn derivatization with phenylisothiocyanate.[47]

Plasma albumin was measured with bromcresol green.[48] The normal/acceptable range was 34–55 g/L. Albumin level in whole saliva was also measured not only as an indicator of salivary gland disease or inflammation, but more importantly, as an index of leakage of plasma constituents into whole saliva. The measurement was by radial immunodiffusion (RID) using the BIND A RID™kit (product codes RN 148.3, RK148; The Binding Site, Ltd., Birmingham, United Kingdom). The values were expected to be significantly much lower in periodontally healthy individuals than in those with gingival inflammation and/or periodontitis.[49]

Parasitemia was estimated from thin films of venous blood following staining with Giemsa and reticulocyte counts made from stained blood smears (0.3% [v/v] brilliant cresyl blue in methanol).

Statistical analysis. Results are expressed as the mean ± SD. When comparing two situations, statistical differences were assessed using a paired *t*-test. For comparison of more

TABLE 1
Anthropometric data of the study population*

Item†	Rural communities‡ (n = 588)	Noma group (n = 36)
Age, years (mean ± SD)	6.38 ± 0.96	5.96 ± 2.62
WAZ % <−2.0 SD	35.8	37.2
HAZ % <−2.0 SD	44.8	42.7
WHZ % <−2.0 SD	10.2	19.4

* Noma = cancrumoris.
† WAZ = weight-for-age Z score; HAZ = height-for-age Z score; WHZ = weight-for-height Z score.
‡ The children were drawn from rural communities in Lagos, Oyo, Osun, Ogun, and Sokoto States in Nigeria.

than two means, an analysis of variance of repeated measurements was used. The level of significance chosen was $P < 0.05$.

RESULTS

General findings. The health histories obtained from the parents/guardians suggested that as many as 60–70% of the impoverished children in Sokoto State residing in less readily accessible rural communities were not vaccinated against measles and the prevalent childhood diseases. This was confirmed by officials in the State's Ministry of Health and attributable mainly to lack of adequate resources in recent years. Malaria (mainly *Plasmodium falciparum* infection) was quite common and often severe in the village children. Blood smears from 90 randomly selected primary school children in impoverished Sokoto rural settings and the affluent urban children showed that 38% and 7.2% of the rural and affluent children, respectively, had malarial parasitemias, with a much higher mean parasite density in the former group. Dietary history obtained in this study confirmed a widespread cultural practice in the village communities of offering any meat products available in the household preferentially to the male head of the family, and only very sparingly to the children and their mothers. The rural children in Sokoto State were usually breast-fed until about the age of two years and weaned to energy-poor millet and corn-based diets, which were occasionally eaten with a vegetable soup. Foods for the infants and children studied were usually prepared under less than adequate hygienic conditions, and invariably in quantities large enough to meet the needs of several meals and/or days. The foods were stored without refrigeration, a situation that favored heavy microbial contamination and growth as previously noted in culturally similar Nigerian communities.[50]

In the village communities studied, the percentages of children (mean ± SD age = 6.38 ± 0.96 years) without noma or any overt illness, who were underweight, stunted, or wasted as reflected by the weight-for-age, height-for-age, and weight-for height Z scores < −2.0 SD were 35.8%, 44.8%, and 10.2%, respectively (Table 1). Equivalent values for an age-matched group of children with noma from similar communities were not different except for a much higher number of wasted children (weight-for-height Z score) in the noma group. Most of the children with noma presented with variable degrees of edema of the limbs, angular stomatitis, discolored and sparse/fluffy hairs, splenomegaly, and hepatomegaly, which were features commonly encountered in protein-energy malnutrition.

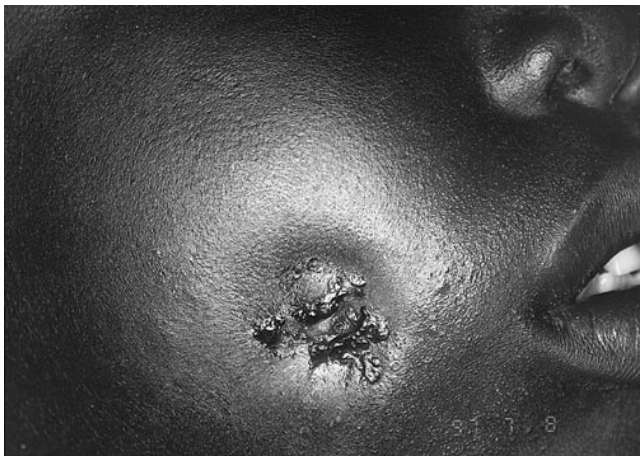

FIGURE 1. A six-year-old boy with a swollen right cheek showing some of the early external signs of cancrum oris (noma) before massive destruction of the facial structures. Intraoral examination revealed extensive necrosis of the gingival tissues and alveolar bone.

Noma (cancrum oris). During a period of less than six months in 1996, our research group and a joint Dutch-German team of plastic surgeons saw 86 new cases of noma in the central location in Sokoto City. In September 1997 alone, 43 additional new cases were seen in the same location. These noma cases came from the remote rural communities in response to prior appeals made to them through the local village chiefs and primary health care workers by the Ministry of Health. Although no age was exempt, most of the noma victims seen were less than 13 years of age, and with virtually equal representation between males and females. Foetor oris was a very prominent feature of the children with noma, and many of them presented simultaneously with extra-oral, well-demarcated gangrenous lesions, most frequently in the scalp and perineum.

Figure 1 shows one of the earliest stages of noma seen in the communities. This six-year-old child, who shared his sleeping room with goats and other domestic animals, presented with a pus-discharging sinus on a swollen right cheek. The lesion had a cone shape with an intra-oral base, and thus, the externally visible aspect did not correlate with the extensive intra-oral destruction of tissues. Most of the new noma cases were seen by us at their most advanced stages when the facial structures were grossly mutilated. Figure 2 shows a girl approximately 3–5 years old with orofacial gangrene involving the right alar of the nose. Intraorally, there was extensive destruction of the alveolar bone and the hard palate. Like the other child (Figure 1), this girl lived in close proximity to rams, donkeys, cattle, and other animals. In both children, as in many others seen, the orofacial necrosis was preceded by a recent attack of measles, and an episode of severe fever that we believed was due to malaria.

Free amino acids in plasma. Table 2 summarizes the mean concentrations of plasma free amino acid levels in

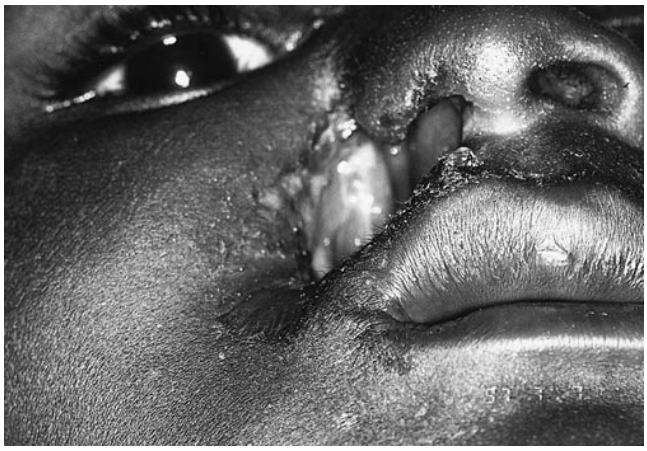

FIGURE 2. An approximately three-year-old girl presenting with facial destruction due to cancrum oris (nona) involving the right alar of the nose, and extensive destruction of the hard palate and the alveolar bone of the right quandrant of the maxilla.

healthy well-fed controls in comparison with levels in impoverished village children without noma or any overt illness and in children with noma. The plasma levels of most of the dietary essential amino acids (EAA), particularly Thr, Val, Leu, Phe, Trp, Arg and Tyr, were significantly reduced in the latter two groups, with hardly any changes in the concentrations of nonessential amino acids (NEA). This resulted in a prominent reduction in the EAA/NEA ratio (%) from 66.9 in the well-fed controls to 51.2 and 47.7 in the rural community (without noma) and noma children, respectively.

Other biochemical parameters. Mean plasma/serum concentrations of retinol, total ascorbic acid, zinc, and albumin were markedly reduced in the impoverished rural children with and without noma, compared with levels in the healthy, well-fed, control children (Table 3). In contrast, whole saliva levels of albumin and cortisol, as well as plasma cortisol concentrations, were significantly lower in the healthy control children than levels in the other two groups. No healthy control child was found with a plasma vitamin A level less than 0.35 μmol/L, and only 8% of this group of children had plasma retinol levels less than 1.05 μmol/L. In contrast, 8% and 20% of the rural children and noma group, respectively, had plasma retinol values less than 0.35 μmol/L. A total of 86.7% of the children with noma had retinol levels less than 1.05 μmol/L. Similarly, it was observed that 77% of the children with noma had plasma zinc concentrations less than 10.8 μmol/L, compared with 54% and 6% for the rural children without noma and healthy controls, respectively. Mean plasma ascorbate levels in the rural and noma groups were in the range consistent with marginal or moderate risk of developing clinical deficiency signs (Table 3). Similarly, the mean serum albumin levels in these two groups were low (less than 34 g/L). Interpretation of whole saliva cortisol concentrations must take into consideration the prominently increased saliva albumin levels in the children with noma and the rural children, a finding suggestive of contamination of saliva cortisol by plasma cortisol from inflammatory exudates. Table 3 also shows that among the socioeconomically deprived children, noma promoted further significant reduction in plasma retinol and zinc concentrations compared with concentrations in the rural children without noma.

DISCUSSION

Health and disease are a reflection of a community, its behavior, and its environment. Previous studies had suggested that poverty was the most important risk indicator for noma in sub-Saharan Africa,[3] with chronic malnutrition, very poor environmental sanitation, an unsafe water supply, and increased exposure to viral and bacterial infections as major predisposing factors.[1–3,10] Findings in our study (Tables 1, 2, and 3) confirmed the strong association between malnutrition and noma in Nigerian children. The plasma free amino acid profiles in the impoverished Nigerian village children studied and in the noma patients (Table 2) were consistent with the syndrome of protein-energy malnutrition (PEM).[51] In the interpretation of our biochemical data, it must be underscored that infections (e.g., malaria, measles) and chronic diseases, as well as trauma, could elicit negative effects on the plasma levels of micronutrients such as ascorbate, ''α-tocopherol, retinol, zinc, and iron.[42,52,53] It is well established that retinol, zinc, and iron are among the negative acute-phase reactants whose plasma levels decrease in response to infection.[52] The latter might explain the observation of the significant reduction in plasma retinol and zinc concentrations in children with noma compared with the other impoverished children without noma (Table 3), although we would not exclude the potential contribution of infection-induced anorexia.

Most of the children with noma showed very marked depletion of plasma retinol, zinc, and ascorbate (Table 3). It was possible that impaired feeding in these sick children further aggravated the already precariously low circulating levels of these micronutrients as seen in the rural children without noma (Table 3). Nonetheless, plasma concentration of retinol lower than 0.7 μmol/L was reflective of very low dietary intake,[53] and levels of approximately 0.35 μmol/L or less indicated a dangerously depleted hepatic store of the vitamin.[54] Most of our patients with noma and many of the village children without noma were in a precarious situation with respect to vitamin A status (Table 3). Surprisingly, xerophthalmia was not a problem in these children, an observation that is consistent with findings in Zairian children by Donnen and others.[55] In that study, 20% of 293 subjects had dedicient serum retinol levels (< 0.35 μmol/L), but no cases of xerophthalmia were encountered. Additionally, the plasma zinc levels found in the children with noma (Table 3) were in the range usually associated with biochemical and clinical alterations resulting from zinc depletion.[45]

Some reports have examined the cellular and molecular effects of nutrient deficiencies,[56] particularly with reference to diseases of the oral cavity;[57,58] therefore, these will not be detailed here. Vitamin A and zinc deficiencies in the human are characterized by diminished cell-mediated immunity, as well as an early breakdown in the integrity of epithelial tissues, including pathologic alterations of the oral mucosa.[53,56] There are, however, some experimental animal studies that suggest that the major impact of vitamin A deficiency is on humoral immune response capability.[59] Among other effects, ascorbate deficiency impairs phagocytosis,[56] and some re-

TABLE 2
Plasma amino acid levels in the study population*

Amino acids†	Healthy controls (n = 12)	Rural community (n = 12)	Noma children (n = 15)
Threonine	118.7 ± 23.4	81.5 ± 15.2‡	67.9 ± 26.0‡
Valine	205.7 ± 28.9	162.5 ± 16.5‡	152.6 ± 10.5‡
Methionine	27.4 ± 3.6	22.8 ± 3.7	18.8 ± 4.1‡
Isoleucine	59.1 ± 9.6	52.2 ± 9.0	49.2 ± 11.2
Leucine	115.3 ± 11.9	77.8 ± 11.7‡	65.8 ± 12.8‡
Phenylalanine	77.3 ± 6.7	51.8 ± 6.1‡	48.2 ± 8.0‡
Tryptophan	49.9 ± 6.0	38.7 ± 10.0‡	27.8 ± 8.2‡
Lysine	125.6 ± 15.8	111.8 ± 22.2	102.3 ± 26.8
Histidine	78.2 ± 2.5	69.4 ± 13.0	64.5 ± 18.0
Arginine	143.6 ± 18.0	102.6 ± 18.3‡	83.1 ± 14.2‡
Tyrosine	60.8 ± 8.5	46.7 ± 5.8‡	44.5 ± 7.8‡
Aspartic acid	33.0 ± 3.3	14.5 ± 6.2‡	9.3 ± 6.0‡
Glutamic acid	131.7 ± 25.9	205.5 ± 61.9	187.6 ± 52.1
Hydroxyproline	19.0 ± 11.7	20.3 ± 4.6	21.2 ± 9.3
Serine	192.4 ± 15.5	138.2 ± 15.6‡	120.6 ± 20.2‡
Asparagine	76.3 ± 9.8	63.4 ± 3.0	65.9 ± 11.1
Glycine	354.1 ± 71.5	309.3 ± 19.4	304.8 ± 18.9
Taurine	141.0 ± 31.3	151.3 ± 46.2	143.6 ± 29.2
Citrulline	31.9 ± 7.1	25.1 ± 4.1	23.6 ± 9.9
Alanine	367.5 ± 66.2	413.1 ± 62.0	395.6 ± 72.2
Proline	173.0 ± 38.2	189.9 ± 13.4	178.9 ± 19.7
Ornithine	67.4 ± 22.1	74.7 ± 21.7	66.7 ± 25.0
ΣEAA	1,061.4 ± 84.9	817.8 ± 106.3‡	724.7 ± 128.6‡
ΣNEA	1,587.2 ± 215.3	1,596.2 ± 108.3	1,517.8 ± 115.6
EAA/NEA (%)	66.9	51.2‡	47.7‡

* Noma = cancrum oris. Data are expressed as the mean ± SD in μmol/L.
† ΣEAA = sum of essential amino acids (includes histidine, arginine, and threonine); ΣNEA = sum of nonessential amino acids.
‡ Significantly different from control ($P < 0.05$).

ports link vitamin C status with antibody response to viral antigens, lymphocyte blastogenesis, and circulating levels of the immune modulator C1q.[38] Protein deficiency in the impoverished rural children with and without noma (Table 2) would impair various parameters of specific and nonspecific immunity.[56,60]

There are many questions to be answered as to the altered host immunologic responses in the development of noma. The observation of some patients with damage, scarring, and fibrosis following noma suggests that there may be a limitation of lesion development. Whether the halt of lesion development is due to a positive shift in immunity as a result of nutritional rehabilitation or some immune recovery following elimination of some other infections or a combination of both or other factors needs investigation. There appears to be no protective immunity that develops after healing,[61] and if any local immunity is developed, it would seem to be of short duration.[62] Since these infections are polymicrobic in nature, it will be difficult to determine the vast array of immune responses to various virulence factors that may participate in lesion development. Only after determining the relevant pathogenic microorganisms and their virulence factors can specific immune responses involved in humoral and cell-mediated immunity be determined. Local and systemic immunosuppression as a result of nutritional and microbial factors also merit investigation. In ANG, a putative precursor for noma, the reported host immunologic responses include leukocyte dysfunction, reduced proliferative response of lymphocytes to phytohemagglutinin, increased T suppressor cells as well as a decreased ratio of T helper/T suppressor cells, and increased serum antibodies to specific anaerobic microorganisms.[18,20] Although chronic malnutrition and viral (measles, HCMV) infections are prominent in the pathogenesis of ANG in impoverished Nigerian

TABLE 3
Circulating levels of retinol, zinc, ascorbate, albumin, and free cortisol in the study population*

	Healthy control (n = 30)	Rural community (n = 32)	Noma patients (n = 36)
Age (years)	6.34 ± 1.41	6.11 ± 1.70	5.96 ± 2.62
Vitamin A (μmol/L)	1.84 ± 0.58	1.38 ± 0.25†	0.92 ± 0.55†‡
Ascorbate (μmol/L)	29.78 ± 6.44	16.62 ± 8.10†	14.32 ± 9.36†
Zinc (μmol/L)	15.94 ± 3.91	12.88 ± 2.16†	9.10 ± 1.94†‡
Serum albumin (g/L)	42.50 ± 5.17	32.40 ± 4.59†	29.50 ± 4.14†
Saliva albumin (mg/L)	19.16 ± 10.08	53.97 ± 20.79†	97.33 ± 27.66†
Plasma cortisol (nmol/L)	300.12 ± 71.85	500.55 ± 111.77†	521.89 ± 102.94†
Saliva cortisol (nmol/L)	3.05 ± 0.95	8.77 ± 3.32†	11.68 ± 6.68†

* Healthy control children were from more affluent, well-nourished homes. The rural children were from the same socioeconomic background as the noma children but had no overt illness. Noma = cancrum oris. Values are the mean ± SD.
† Significantly different from the health control values.
‡ Significantly different from levels in rural children without noma.

children,[2,22] only a relatively small percentage of the highly prevalent ANG cases evolve into noma.

The significantly elevated plasma levels of free cortisol noted in the deprived Nigerian children with or without noma (Table 3) is a well-documented feature of protein malnutrition[63] and may be due in part to concurrent infections.[64] Consistent with the well known existence of a strong linear correlation between free cortisol in blood and saliva cortisol levels,[65,66] whole saliva contents of cortisol were markedly elevated in the deprived children with or without noma, in comparison to the healthy controls (Table 3). However, there was evidence of blood contamination of whole saliva in the former groups as reflected in the significantly elevated saliva levels of albumin. The latter notwithstanding, our studies demonstrated increased oral burden of a potent immunosuppressive hormone in children with noma or at risk for the disease. Individuals under steroid-mediated immunosuppression are known to have increased risk of episodic reactivation to primary viral infection or reinfection.[67] It is perhaps of some relevance that administration of glucocorticoids has been claimed to induce ANG and noma-like lesions in traumatized experimental animals.[68]

We had earlier reported that HCMV and possibly other herpesviruses such as the Epstein-Barr virus-1 and herpes simplex virus,[22] as well as the measles virus,[2] might contribute to the onset and/or progression of ANG in malnourished Nigerian children. In the present study, as in others,[1,2] virtually all the children with noma reported some debilitating infections in the recent past, with measles being the most common. Cases of noma seen by Tempest[1] occurred most frequently during the so-called hungry months in Nigeria, which were also the months when the incidence of measles was highest in the communities. The peak incidence of a severe viral illness often appears concurrently or immediately preceding that of bacterial infections.[69] Viral infections of the respiratory tract, for example, predispose the latter to bacterial superinfections and this is attributed to generation of bacterial binding sites by virus-induced changes in cell-surface glycoconjugates and expression on the eukaryotic cell membrane of viral proteins that can act as bacterial receptors.[69] The measles virus not only down-regulates IL-12 needed to drive T helper cell response towards Th-1 dominance,[15] but also infects and damages epithelial tissues.[70] Earlier studies had shown that malnourished Nigerian children with severe attack of measles, were prone to develop deep erosive ulcers of the mouth and eyes,[16] which were often secondarily infected by the herpes simplex virus, and these children were found to develop noma later.[10] Among the herpesviruses, HCMV is the one most frequently associated with immune suppression,[71] and one of the most important ulcerogenic viruses in immunosuppressed individuals.[72] Generally, as many as 85–95% of deprived children in developing countries acquire HCMV by five years of age,[73] and hypercortisolemia in such children (Table 3) could reactivate latent viruses. These infections also promote malnutrition.[74]

We had previously reported recovery of *F. necrophorum, P. intermedia,* alpha-hemolytic streptococci, and *Actinomyces* spp. from 88%, 75%, 50%, and 38% of our Nigerian noma cases, respectively.[28] This first reported isolation from human noma lesions of *F. necrophorum,* an anaerobic gram-negative, nonspore-forming pathogen primarily associated with animal diseases, might have important etiologic and animal transmission implications, particularly in view of the residential closeness of the children with noma and domestic animals. *Fusobacterium necrophorum* is considered a key constituent of a mixed infection that causes foot-rot, a foul smelling necrotic disease involving the hooves and surrounding tissues in livestock.[75,76] There are marked similarities between foot-rot in ungulates as well as necrobacillosis in wallabies and related species, and noma in humans. These lesions are characterized by rapid tissue necrosis, abscess formation, and a very putrid smell.[1,10,28,77] The main pathogen involved in animal necrobacillosis is *F. necrophorum* (biovar A strains), and its infective dose is drastically reduced by the concurrent presence of other bacterial species particularly *Escherichia coli, Staphylococcus aureus, Bacteroides* species, peptostreptococci, and various aerobes and facultative anaerobes.[78] The periodontal tissues and face are the sites most commonly affected by necrobacillosis in wallabies,[25,79] and studies show that *F. necrophorum* is the predominant microorganism occurring in 69% of the lesions.[26] At this stage, we have not confirmed whether the strains of *F. necrophorum* isolated from human noma are similar to the animal biovars. *Fusobacterium necrophorum* has little ability to invade intact epithelium, and infection usually arises from contamination of damaged mucosa following ulceration/abrasions, trauma, or a debilitating condition.[25,80]

Based on our previously published microbiologic data,[22,28] and the present biochemical findings, we have proposed a tentative scheme to explain the pathogenesis of noma in deprived Nigerian children (Figure 3). As indicated in the scheme and extensively reported in the literature,[56,74,81] a complex three-way relationship exists between malnutrition, immune dysfunctions in the host, and increased susceptibility to infections. Viral infections are more severe in the malnourished,[74,82] and Beck[81] has shown that malnutrition can alter the genotype of a virus, resulting in a more potent virus. The interactions between viral infections, malnutrition, and diminished host resistance result in impaired oral mucosal immunity, local viral multiplication in the oral tissues, and selective growth of pathogenic bacteria.[22,27,56,57] Endocrine function adaptations in malnourished African children usually include increased secretion of growth hormone, cortisol, and norepinephrine, with decreased production of insulin and the thyroid-stimulating hormone.[83,84] The mean plasma cortisol level in the well-fed control Nigerian children was within the lower range for published normal morning values[85], and significantly much lower than the mean levels in the impoverished groups with and without noma (Table 3). The increased plasma concentrations of free cortisol in the impoverished children could be attributed in part to infections.[86] Some infections, particularly gram-negative bacteria, viral, and fungal infections, are more frequent and severe in glucocorticoid-treated patients.[20,87] There are suggestions that chronically elevated basal endogenous glucocorticoid levels, even at concentrations that might appear within the physiologic range as observed in the impoverished children (Table 3), promote a shift from a type 1 to a type 2 cytokine production profile.[88,89] Glucocorticoid excess enhances replication of a number of latent viruses,[90–92] and promotes increased susceptibility of the oral mucosa to viral infections.[91] Increased steroid levels in the mouth could also serve as a

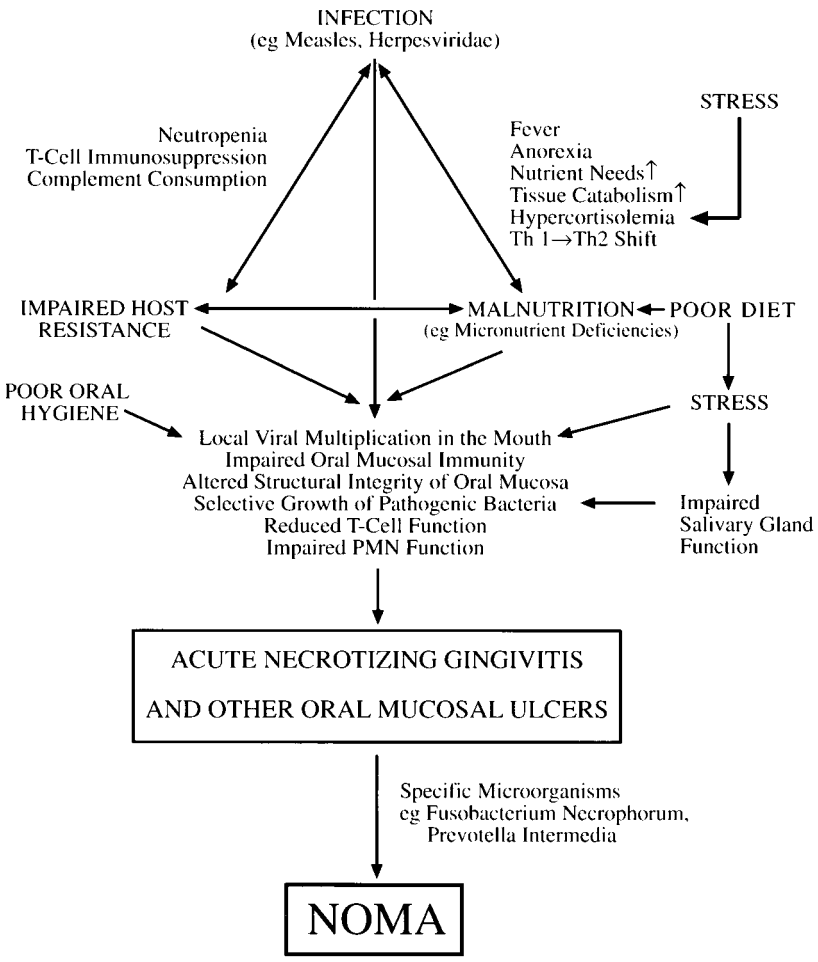

FIGURE 3. Proposed schematic representation of the pathogenesis of cancrum oris (noma). Th = T helper cell; PMN = polymorphonuclear neutrophils.

rich nutrient source for anaerobes.[20] Stress also impairs salivary gland function, reducing the volume of saliva and the availability of salivary proteins, which are protective against the growth and pathogenicity of oral microflora.[57,93] Studies of the effect of malnutrition (PEM) on oral microbial ecology in Nigerian village children have demonstrated prominently increased recovery of spirochetes and anaerobic rods compared with control groups of well-fed children from the same ethnic background.[27] Cellular depletion of key nutrients such as zinc, retinol, ascorbic acid, and the essential amino acids, will impair the structural integrity of the oral mucosa,[45,52,53,56] thus creating easy portals of entry for the pathogenic microorganisms and their products.[17,57]

Our scheme (Figure 3) proposes that viral infections acting in concert with malnutrition and the resulting selective overgrowth of pathogenic periodontal bacteria play a key role in the genesis of ANG in impoverished Nigerian children. There are some striking similarities between aspects of the proposed scheme (Figure 3) and the stages leading to corneal ulceration in vitamin A-deficient children infected by measles. In the latter, it is suggested that immunosuppression elicited by the local proliferation of the measles virus in the eye might trigger the invasion of pathogenic microbes, which further damage the cornea whose structural integrity is already compromised by retinol deficiency and lesions of measles keratitis.[17] Why a relatively few malnourished children with ANG should progress to noma and the great majority do not is still unclear. Jelliffe[94] has proposed that an unknown factor X is responsible for the progression of ANG and/or other oral mucosal ulcers to noma. It is our hypothesis that contamination by a consortium of microorganisms, of which *F. necrophorum* is an important member, constituted the factor X.[3,28]

When human noma-like lesions occur in the periodontal tissues and face of wallabies, the main pathogen is *F. necrophorum,* often with other microorganisms such as *F. nucleatum, Bacteroides* species, peptostreptococci, various aerobes, and facultative anaerobes, which increase its infectivity.[26,78,79] *Fusobacterium necrophorum* has little ability to invade intact epithelium and infection usually results from fecal contamination of damaged mucous membrane in the animals, especially under conditions of overcrowding and food scarcity.[78–80] It is our speculation that in impoverished Nigerian children with ANG and/or malnutrition-induced impairment of the structural integrity of the oral mucosa, fecally contaminated foods, and household drinking water[50] are ready sources of *F. necrophorum.* This hypothesis, which has received partial validation,[28] is the subject of ongoing studies in

our laboratories. In addition to the classical endotoxin and a dermonecrotic toxin, *F. necrophorum* elaborates several other toxic metabolites capable of causing rapid tissue destruction.[28] It is perhaps not fortuitous that in two Nigerian languages commonly spoken in Sokoto State, noma is commonly referred to as gaude and sadde. In English, both words mean disease of cattle rearers, implying that in the traditional culture, noma is considered a disease transmitted to the people from animals. Prevention of noma in African children will require measures that address the severe problems of malnutrition, increased exposure to infectious diseases, and the rapidly deteriorating sanitation in most impoverished, overcrowded communities.[3] The preventive approach assumes more importance in view of studies suggesting that *F. necrophorum* is weakly immunogenic in animals, an observation that minimizes the prospect of early development of an effective vaccine for control of the disease.[95,96]

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