Chemotaxis, random mobility, and mobilization of polymorphonuclear leucocytes in malnutrition^{1, 2}

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SYNOPSIS Neutrophil mobilization following administration of *Pseudomonas* polysaccharide was significantly reduced in malnutrition, especially during infection. The random mobility of polymorphonuclear leucocytes (PMNs) was slightly decreased in undernutrition. Chemotactic migration of PMNs was depressed and correlated more with the presence of infection than with nutritional deficiency. It is possible that these abnormalities of PMN mobilization and mobility in malnourished individuals contribute to suboptimal amount, kinetics, and pattern of tissue inflammatory response to bacteraemic challenge.

The common association of malnutrition and infection is well known. In the last few years, immunological studies of undernourished individuals have revealed impaired host defence mechanisms (Chandra, 1974a, 1976a, 1976b). The defects involve serum immunoglobulin levels and antibody response to some antigens (Chandra, 1976c), cell-mediated immunity (Geefhuysen *et al*, 1971; Smythe *et al*, 1971; Chandra, 1972; McFarlane and Hamid, 1973), thymus-dependent T lymphocytes in the peripheral blood (Chandra, 1974b; Ferguson *et al*, 1974), oxidative metabolism and bactericidal capacity of polymorphonuclear leucocytes (PMNs) (Selvaraj and Bhat, 1972; Seth and Chandra, 1972), complement (Sirisinha *et al*, 1973; Chandra, 1975a), serum siderophilin (Antia *et al*, 1968), and secretory antibody response (Chandra, 1975c). Similar abnormalities have been noticed in low-birth weight infants who are small for gestational age as a result of fetal malnutrition (Chandra, 1975b, 1974c; Ferguson *et al*, 1974).

We have studied another parameter of PMN function, namely, neutrophil mobilization, random and chemotactic mobility, and correlated it with nutritional status and the presence of infection.

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Material and Methods

PATIENTS AND CONTROLS

Four groups of children in the age group 1-4 years were studied. Group I consisted of 10 malnourished patients with infection, group II, eight malnourished children without concurrent infection, group III, 10 well nourished children with infection, and group IV, 10 healthy controls. Protein-calorie undernutrition was diagnosed on the basis of history of reduced dietary intake, clinical features such as growth failure, loss of subcutaneous tissues, and hair changes, and anthropometry. Body weight and height were between 50 and 70% of the 50th percentile for age on Harvard growth standards. None of the children showed oedema. Infection was documented on clinical and microbiological evidence. The diagnoses included bacterial diarrhoea and dysentery, pneumonia (6 cases each), meningitis (2 cases), otitis media (2 cases), abscess in the abdominal wall (2 cases), and extensive pyoderma and infected scabies (one case each). Enteropathogenic *Escherichia coli*, *Klebsiella*, and *Staphylococcus aureus* were isolated in three instances each, *Streptococcus haemolyticus*, *Salmonella typhi*, and *Pseudomonas pyocyanea* in two instances each, and *Streptococcus faecalis* and *Diplococcus pneumoniae* once each. In a few cases, the qualitative nitroblue tetrazolium test (Feigin *et al*, 1971) supported the presence of bacterial infection.

The data were analysed by Student's *t* test of statistical significance.

LEUCOCYTE TESTS

Random mobility was measured by the method

described by Bryant *et al* (1966). Cell suspensions containing 5×10^6 PMNs per ml of 10% pooled normal human serum were placed in vertically positioned microhaematocrit tubes. Cell movement from the point of origin to the advancing front of migrating cells was measured after 4 hours with an ocular micrometer.

Chemotaxis was evaluated by Miller and Nilsson's (1970) method. A suspension containing 5×10^6 PMNs per ml was placed on one side of a 3μ pore membrane filter (Millipore Corporation). Chemotactic factor generated from fresh serum by incubation with *E. coli* was placed on the other side of the filter. After incubation at 37°C for 3 hours, the average number of PMNs per high-power field reaching the lowest surface of the membrane was determined.

Mobilization of PMNs from the bone marrow was assessed by two methods: (1) by giving 0.15 ml of 1:1000 aqueous adrenaline solution intravenously and counting the number of neutrophils in the blood just before and at 2, 5, 10, and 20 minutes after administration; (2) by an intravenous injection of 0.05 μ g per kg of body weight of Piromen (*Pseudomonas* polysaccharide) and determining the absolute neutrophil count in the blood at one-hour intervals for 6 hours.

Results

NEUTROPHIL NUMBER

The distribution of cases by peripheral blood neutrophil count is given in table I. One child each in groups I, II, and III had significant neutropenia; in two, the diagnosis was typhoid fever, confirmed by microbiological isolation of the organism. A marked increase in total leucocyte count and neutrophils > 6000 per mm^3 was observed in five of 10 malnourished infected patients and in seven of 10 well nourished children with infection.

NEUTROPHIL MOBILIZATION

Adrenaline stimulation produced similar increases in absolute neutrophil count in all the groups (table II). Piromen caused a rise in body temperature in all children. There was a marked increase in the neutrophil count of peripheral blood in healthy children (table II); malnourished patients with infection showed a poor response, and groups II and III had intermediate values.

PMN RANDOM MOBILITY

Spontaneous random migration was reduced slightly in malnourished children without concurrent infection (table III). The presence of infection did not

Group	Number	Neutrophils per mm^3		
		<2000	2000 to 6000	>6000
I Malnourished and infected	10	1	4	5
II Malnourished, no infection	8	1	6	1
III Well nourished, with infection	10	1	2	7
IV Healthy	10	0	9	1

Table I Leucocyte counts

Group	Mean Increase in Neutrophils (per mm^3) after		Statistical Significance (P) ¹ of Difference from			
	Adrenaline	Piromen ¹	Group I	Group II	Group III	Group IV
I Malnourished and infected	2183	1075	—	<0.05	<0.01	<0.001
II Malnourished, no infection	2565	2117	<0.05	—	NS	<0.01
III Well nourished, with infection	2098	2630	<0.01	NS	—	<0.01
IV Healthy	2750	6853	<0.001	<0.01	<0.01	—

Table II Maximum increase in absolute neutrophil count following adrenaline or Piromen

¹P values for data on Piromen only

NS = not significant

Group	Random Mobility (mm)		Statistical Significance (P) of Difference from			
	Range	Mean	Group I	Group II	Group III	Group IV
I Malnourished and infected	8-13	10	—	NS	<0.05	<0.05
II Malnourished, no infection	9-14	11	NS	—	<0.05	<0.05
III Well nourished, with infection	12-20	17	<0.05	<0.05	—	NS
IV Healthy	15-22	18	<0.05	<0.05	NS	—

Table III Random mobility of polymorphonuclear leucocytes

NS = not significant

make any appreciable difference in this function.

PMN CHEMOTAXIS

Directed non-random migration was profoundly depressed in malnourished children with infection (table IV). Infection was the apparent determining factor for this abnormality since the well nourished infected group also showed a significant reduction of PMN chemotaxis.

Discussion

Our observations indicate that mobilization and movement of PMNs may be significantly impaired in malnutrition, especially in the presence of infection. The data provide an explanation for the type and intensity of inflammatory response mounted by an undernourished individual challenged by a pathogen.

The poor neutrophil response of malnourished children with *Pseudomonas* polysaccharide indicates impaired marrow release. In severe kwashiorkor, concurrent infection may elicit little or no leucocytosis (Trowell *et al*, 1954). Asirvatham (1948) induced turpentine abscesses in protein-deficient rats and found no leucocytic response. The bone marrow was atrophic. The abnormalities were reversed on refeeding. A slowing down of cell generation and tissue proliferation is a basic alteration of the organism in response to protein deficiency. Bone marrow with its rapid cell turnover is no exception (Sood *et al*, 1965). In protein-deficient rabbits, intravenous administration of *Staph. aureus* elicited a poor and transitory neutrophil response, the bacteria persisted and multiplied in the blood and tissues, the lesions were necrotic rather than localized abscesses, and there was an early high mortality (Bhuyan and Ramalingaswami, 1972).

Additionally, endotoxaemia associated with infection may deplete the marrow PMN reserves. Endotoxin is frequently detected in the blood of malnourished children (R. Suskind, personal communication).

Neutrophil response following adrenaline administration was comparable in all the four groups studied. The slight differences in the mean absolute

increase in PMNs of malnourished and healthy children were not statistically significant. Thus, no change in the non-marrow or marginal pool of neutrophils was demonstrable.

There are two basic types of movement shown by PMNs—random, unstimulated mobility, and non-random migration directed to a chemotactic stimulus. In malnourished children, random mobility was slightly reduced; the differences were statistically significant at the 5% level of probability. It is possible that this defect is due to changes induced by protein-calorie deprivation in the cell membrane, the amount of contractile actin in the microfilaments or in its polymerization ability. Chemotaxis was reduced maximally in malnourished patients with concurrent infection. It is possible that both infection and undernutrition contribute to this impairment, though the role of the former was obviously much more significant since well nourished children with infection (group III) also showed a marked reduction in chemotactic migration (table IV). Data from another group are in conformity with our observations on chemotaxis (Rosen *et al*, 1975). The kinetics of the chemotactic response are significantly altered in kwashiorkor (Steven Douglas, personal communication). Preliminary data obtained by E. R. Stiehm (personal communication) indicate that the generation of chemotactic factor(s) from the serum of healthy and malnourished subjects is comparable.

It is possible that in malnourished individuals abnormalities of PMN mobilization and mobility described above contribute to the suboptimal quantity, kinetics, and pattern of tissue inflammatory response to bacteraemic challenge.

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Group	Chemotaxis (% of mean value for healthy control)		Statistical Significance (P) of Difference from			
	Range	Mean	Group I	Group II	Group III	Group IV
I Malnourished and infected	16-48	28	—	0.01	0.08	0.001
II Malnourished, no infection	56-96	72	0.01	—	0.05	0.06
III Well nourished, with infection	28-56	44	0.08	0.05	—	0.01
IV Healthy	—	100	0.001	0.06	0.01	—

Table IV Response of polymorphonuclear leucocytes to chemotactic stimulus

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