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PHAGOCYTE FUNCTION IN PROTEIN-CALORIE MALNUTRITION

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SUMMARY

Sixteen children with the kwashiorkor type of protein-calorie malnutrition (PCM) have been investigated in an attempt to assess the functional activity of their peripheral blood phagocytes. Hexose-monophosphate shunt (HMS) activity in the resting and phagocytizing state and bactericidal capacity with *Escherischia coli* and *Staphylococcus aureus* were measured. The findings indicate HMS stimulation in the normal range as determined by $^{14}CO_2$ production during phagocytosis. The bactericidal capacity of phagocytes from kwashiorkor patients was normal in the early phase (first 30 min) and significantly reduced during the later phase (after 60 min) of the assay as compared with cells from healthy children.

INTRODUCTION

Infectious diseases are a major cause of death in children with severe PCM (Scrimshaw, Taylor & Gordon, 1968). However, the pathophysiology of the development of infections in malnutrition is poorly understood. Several reports from different parts of the world where infantile malnutrition is prevalent, have indicated an impairment in cell-mediated immunity (Smythe *et al.*, 1971; Geefhuysen *et al.*, 1971; Sellmeyer *et al.*, 1972) and reduced serum complement levels (Sirisinha *et al.*, 1973). The results concerning humoral immunity are conflicting (Scrimshaw *et al.*, 1968; Watson & Freeseman, 1970; Reddy & Srikantia, 1964; Cohen & Hansen, 1962). In an attempt to assess the status of the afferent limb of the immune response we have studied peripheral blood phagocyte function—HMS stimulation during phagocytosis (Karnovsky, 1968) and bactericidal capacity (Quie *et al.*, 1967)—in a group of children with well-defined kwashiorkor.

PATIENTS AND METHODS

Sixteen African children between the ages of 10 and 30 months, admitted to the Pediatric

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Department of the Centre Hospitalier de l'Université de Treichville in Abidjan, Ivory Coast, because of severe PCM, have been studied. All of the children investigated showed the typical kwashiorkor syndrome of PCM using anthropometric, clinical and biochemical criteria as established by Jelliffe (1966). None of the patients had overt clinical infection or received antibiotics. Total serum proteins were measured by the Biuret method, the serum protein fractions were determined by electrophoresis on cellulose acetate strips. Healthy children from a village near the Nestlé Foundation Laboratory have been studied as a control population. Phagocytic indices for monocytes were determined using antibody-coated erythrocytes and polystyrene particles (Douglas *et al.*, 1972).

Stimulation of the HMS was measured using a whole blood assay of $^{14}CO_2$ production measured with a Bactec 301 radiometric detector (kindly provided by Johnston Laboratories Incorporated, Cockeysville, Maryland) (Keusch & Douglas, 1973). Whole blood was incubated with [1-^{14}C]glucose and latex particles (phagocytizing cells) and without latex (resting cells). Values were corrected for the number of phagocytes.

The bactericidal assay was performed as previously described (Douglas, Davis & Fudenberg, 1969). The leucocytes were separated by dextran sedimentation. The phagocytes (polymorphonuclear and monocytes) were counted and incubated in Eagle's Minimal Essential Medium with *E. coli* (ATCC 10536) or *S. aureus* at a ratio of five to ten bacteria per cell. Fresh serum pools from either kwashiorkor patients or control children were added to the assay to provide opsonin. In each experiment a control assay was run without phagocytes. Aliquots from the incubation mixture were removed after 0, 15, 30, 60, 120, 180 and 240 min. The cells were then lysed hypotonically to release viable intracellular bacteria. The samples were diluted logarithmically and plated in tryptose soy agar. After 24 hr of incubation the number of bacterial colonies were counted.

Non-lysed cell pellets from the bactericidal assays were fixed in 1·5% glutaraldehyde, post-fixed in osmium tetroxide, dehydrated and embedded in epon. Thin sections were examined with a Siemens 101 electron microscope.

RESULTS

Clinical and laboratory findings

The children with kwashiorkor were apathetic and lethargic, all had severe oedema, hepatomegaly, cold extremities, angular stomatitis, and other mucocutaneous lesions. Moon facies occurred frequently. The anthropometric and biochemical data from the children examined are summarized in Tables 1 and 2. All of the sick children had albumin levels below 2·0 g/100 ml (a mean of 1·5 g/100 ml) which is compatible with the diagnosis of kwashiorkor (Hansen, 1961). The degree of oedema correlated well with the hypoalbuminaemia. The patients had mean a total leucocyte count of 7800/mm^3 ± 2500 with 3700 ± 1800/mm^3 neutrophils and 350 ± 210 monocytes (total phagocytes 4200 ± 1950).

Phagocytic index

The engulfment of polystyrene particles and antibody-coated erythrocytes was comparable for monocytes from kwashiorkor patients and control children (Table 3).

HMS stimulation

$^{14}CO_2$ production from [1-^{14}C]glucose by phagocytes during phagocytosis is shown in

TABLE 1. Anthropometric data

	Kwashiorkor (N 16)	Control (N 16)	*P*
Age (months)	24±6*	21±5	—
Weight (kg)	8·3±1·3	10·49±1·11	
Percentage of normal†	66±7	88±8	$P<0·001$
Height (cm)	82·2±6·8	80·17±4·88	—
Percentage of normal	90–100	90–100	NS
Mid upper arm circumference (cm)	11·78±1·05	14·9±1·11	
Percentage of Normal	72±7	92±7	$P<0·001$
Head circumference (cm)	45·85±1·74	46·75±1·7	—

* Mean±standard deviation.
† Percentage of normal values according to Jelliffe (1966).
NS = not significant.

TABLE 2. Biochemical data

	Kwashiorkor (N 16)	Control (N 16)	*P*
Proteins (g/100 ml)	3·61±0·52*	6·86±0·56	$P<0·001$
Albumin	1·50±0·32	3·93±0·54	$P<0·001$
α_1-globulin	0·17±0·03	0·17±0·07	NS
α_2-globulin	0·36±0·07	0·42±0·13	NS
β-globulin	0·30±0·05	0·61±0·11	$P<0·001$
γ-globulin	1·28±0·33	1·73±0·43	$P<0·005$

* Mean±standard deviation.
NS = not significant.

TABLE 3. Phagocytosis

	Kwashiorkor (N 4)	Control (N 2)	*P*
Polystyrene particles			
Percentage of phagocytic cells	93·4±2·6*	97·3±1·8	NS
Polystyrene particles/ monocyte	25·3±0·7	26·2±1·7	NS
Coated erythrocytes (EA)			
Percentage of phagocytic cells	66·1±27·1	78·5±12·7	NS
EA/monocyte	2·2±0·4	2·0±0·2	NS

* Mean±standard deviation.
EA = sheep erythrocytes coated with Forssman antibody (dilution 1:3200).
NS = not significant.

TABLE 4. $^{14}CO_2$ production from [1-^{14}C]glucose by phagocytosing and resting cells

	Kwashiorkor (N 6)	Control (N 10)	*P*
Δ P−R†	55·62±24·81*	45·81±21·98	NS
Δ P−R (corrected)‡	82·76±46·16	62·80±23·92	NS

* Mean±standard deviation.
† Δ P−R refers to values for phagocytizing minus values for resting cells.
‡ Δ P−R (corrected) for total leucocyte count.
NS = not significant.

Table 4. The findings indicate that the cells from kwashiorkor children showed $^{14}CO_2$ production in the normal range.

Bactericidal assays

Bactericidal assays have been performed using cells from five children with kwashiorkor and five controls (Table 5 and Figs 1 and 2). Table 5 demonstrates the ratios of viable intracellular bacteria in phagocytes from kwashiorkor and control children after various incubation periods. More *E. coli* or *S. aureus* are viable in the cells from five kwashiorkor children than from controls (ratio >1) after 60 and 120 min. The patient (assay 2) whose cells showed the same bactericidal activity as the control child had received a transfusion approximately 12 hr before the blood sampling for the bactericidal assay. Similar results

TABLE 5. Bactericidal assay

		Minutes		
Assay*	Bacteria	30	60	120
	E. coli			
1		1·39†	4·24	5·83
2‡		1·56	0·48	0·26
3		0·93	9·69	5·91
4		3·97	2·56	2·04
5		0·90	1·38	1·91
	S. aureus			
6		0·72	3·76	4·00

* Each assay refers to studies of individual patients and controls.
† Ratio of the number of viable intracellular bacteria in kwashiorkor cells/control cells.
‡ Patient transfused

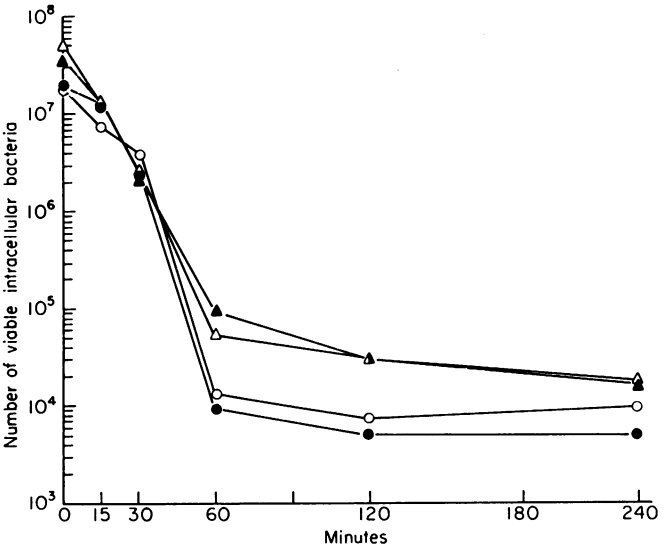

FIG. 1. Bactericidal curves with *E. coli* (penicillin–streptomycin added at 30 min. to kill extracellular bacteria). At 240 min kwashiorkor opsonin pool and normal opsonin pool in the absence of phagocytes showed no killing. (▲) Kwashiorkor cells + kwashiorkor opsonin. (△) Kwashiorkor cells + normal opsonin. (●) Normal cells + kwashiorkor opsonin. (○) Normal cells + normal opsonin.

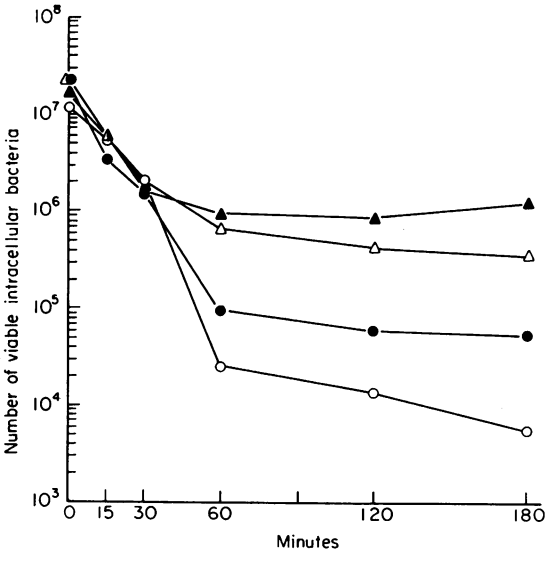

FIG. 2. Bactericidal curves with *E. coli* (▲) kwashiorkor cells and (△) normal cells (no antibiotics added) and *S. aureus* (●) kwashiorkor cells and (○) normal cells (penicillin–streptomycin was added at 30 min).

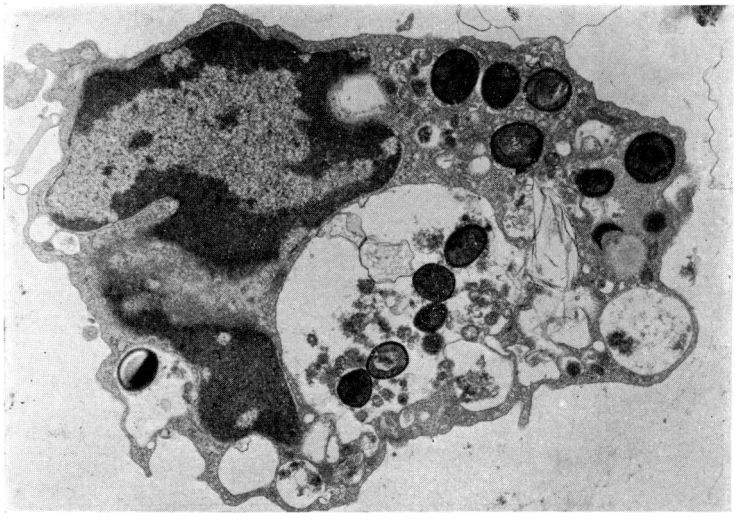

FIG. 3. Electron micrograph of neutrophil obtained from a child with kwashiorkor and incubated for 240 min with *S. aureus*. Bacteria are present with phagocytic vacuoles and extensive degranulation has occurred. (Magnification × 10,000).

were obtained using either kwashiorkor or normal serum pools as opsonin. No bactericidal activity was demonstrated in the opsonin control assay (without phagocytes).

Electron microscopy

Electron microscopy of resting and phagocytizing phagocytes showed no qualitative differences in granules, mitochondria, phagocytic vacuoles or extent of degranulation (Fig. 3).

DISCUSSION

During phagocytosis of polystyrene particles by peripheral blood phagocytes from kwashiorkor children there is normal stimulation of the HMS as measured by $^{14}CO_2$ production from [1-^{14}C]glucose. The phagocytic index for glass adherent monocytes showed no impairment in particle engulfment. Electron microscopic studies of phagocytic cells demonstrated no abnormalities in vacuole formation or the extent of degranulation. There is, however, impaired bactericidal activity of the phagocytes obtained from children with kwashiorkor; there was reduced killing of *E. coli* or *S. aureus* after 60 min as compared to cells from controls. Our observations differ from those of Selvaraj & Seetharam-Bhat (1972), in children with PCM in India, who reported diminished HMS stimulation during phagocytosis and lower or almost no bactericidal activity throughout the whole incubation period, similar to cells from patients with chronic granulomatous disease (CGD) (Quie *et al.*, 1967).

The demonstration of abnormal bactericidal activity in phagocytes from children with severe PCM may have major implications in understanding the pathophysiology of the increased susceptibility to infectious diseases of these patients. The abnormality in bactericidal

activity is distinct from other known bactericidal defects of phagocytes as in CGD, or in the Chediak Higashi syndrome (CHS). Although severe impairment in bactericidal activity occurs in phagocytes from patients with CGD (Quie *et al.*, 1967; Davis *et al.*, 1968) and CHS (Root, Rosenthal & Balestra, 1972) the relationship between this *in vitro* defect and clinical infections in these patients has not been determined. Furthermore, the observation that heterozygous mothers of CGD patients have a marked *in vitro* bactericidal abnormality and no evident increased propensity to bacterial infections (Douglas, 1970) remains to be explained.

Previous studies of the metabolism of leucocytes from children with PCM have revealed diminished activity of cytoplasmic enzymes (Yoshida, Metcoff & Frenk, 1968) and reduced contents of several metabolites (Yoshida *et al.*, 1967). The possibility that the impaired bactericidal activity which we have observed for phagocytes from children with kwashiorkor during the late phase of the *in vitro* assay is related to substrate depletion, or other unknown biochemical factors requires further investigation. The kinetics of bactericidal activity for phagocytes from children with kwashiorkor, namely diminished killing after 1 hr has not been reported previously. Furthermore, this is the first demonstration of impaired phagocyte function in an environmentally determined disease.

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