Leucocyte Function in Protein Deficiency States

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SUMMARY

Seventy-five patients, comprising 3 groups of 25 normal infants (group I), 25 infants suffering from protein energy malnutrition (PEM—kwashiorkor) (group II) and 25 infants with PEM plus infection (group III) were examined, with particular reference to their total leucocyte count and the response of their white cells to incubation with nitro-blue tetrazolium (NBT), with and without latex particle stimulation. No difference in leucocyte counts could be demonstrated between the groups; however, both groups of malnourished patients (groups II and III) had elevated NBT levels, although no difference could be detected between those malnourished infants with and without clinical or laboratory evidence of infection, or both. The data indicate that although primarily protein-deficient malnourished patients have a quantitative deficiency in leucocyte response, qualitative leucocyte function may be normal.

S. Afr. Med. J., **48**, 528 (1974).

Metchinkoff[1] made the first observation that phagocytosis was an important aspect of leucocyte function and host resistance, and although clinicians have for decades appreciated that adequate numbers of leucocytes were essential for host defence, the concept that adequate function is also important, is of recent origin. During the past 10 years, methods have been developed which allow for the separation of different parameters of leucocyte function, and these methods have been applied to clinical situations. One major step has been the introduction of a supravital staining technique by means of which phagocytosis and intraleucocyte metabolic activity can be measured. Park *et al.*[2] recognised the fact that nitro-blue tetrazolium (NBT) does not normally enter leucocytes unless there is increased leucocyte membrane permeability or pinocytic activity, both of which occur primarily with bacterial infection. The NBT, on entering the active leucocyte, accepts hydrogen ions formed from the increase in glucose oxidative processes and precipitates as a blue intracellular inclusion (formazan). These inclusions can be seen under the light microscope, and the cells containing them counted and expressed as a percentage of total leucocytes present. Increased susceptibility to infection and decreased NBT counts have been described in patients with congenitally abnormal leucocyte function (chronic granulomatous disease of childhood),[3] while the test has also been found useful as an aid in the differential diagnosis of febrile disorders,[4] and to monitor bacterial infection in patients undergoing intravenous or intra-arterial infusions.[5]

As recurrent and severe infections frequently occur in children suffering from severe protein deficiency,[6] this study was undertaken to ascertain whether leucocyte numbers and activity (as measured by the NBT test) were abnormal in these children.

PATIENTS AND METHODS

Three groups of 25 Black infants were studied (Table I).

TABLE I. POPULATION STUDIED

	No. of patients	Mean age (mo)	Mean height (cm)
Group I (control)	**25**	**12**	**70,56**
Group II (PEM)	**25**	**22**	**77,98**
Group III (PEM + infection)	**25**	**18**	**78,14**

Group I comprised the total control group and were defined as normal by their attendance at a well-baby clinic, by having no clinical evidence of malnutrition or any other disease, and by 80% of them having a mass greater than 80% of the 50th percentile for their age. Group II were infants with protein energy malnutrition (kwashiorkor) as defined by the Joint FAO/WHO Expert Committee on Nutrition (1971) without any clinical or laboratory evidence of infection, who were being followed-up as outpatients. Group III were non-anaemic (mean Hb 10,23 g ± 1,6 g) predominantly kwashiorkor inpatients with clinical or laboratory evidence of infection, or both, being present.

All patients were weighed and measured, and their blood, total proteins, albumin and polymorphonuclear leucocyte count estimated. Venous blood was further utilised for incubation with NBT and latex particles as decribed by Park *et al.*[2] and the percentage of neutrophils containing a definite mass of formazan (reduced NBT) was determined. All estimations and slides were performed by the same investigator. Total proteins and serum albumin levels were correlated in each group, according to the following: (*i*) the total leucocyte count; (*ii*) the number of cells containing NBT; and (*iii*) the number of cells containing NBT after stimulation with latex particles, and correlation coefficients calculated. The total leucocyte count and the number of cells containing NBT with and without latex stimulation in the malnourished groups (groups II and III) were each compared with those of the normal group (group I) and with each

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Date received: 5 September 1973.

other, and an analysis of variance leading to the Student's *t*-test performed.

RESULTS

Table II gives the results of the leucocyte count and percentage of cells containing NBT, with and without latex stimulation, while Table III shows the *P* values between the means for leucocyte number and function in the 3 groups. The malnutrition groups (groups II and III) show no increase in leucocyte count; however, their leucocytes have statistically significant elevated activity levels (as measured by the NBT test) compared with the normal group (group I). No difference could be found between those malnourished infants without, compared to those with, infection. Table IV details the correlation between total protein and serum albumin levels and the measured parameters in each group. No significant correlation could be found in any of the groups between total serum protein or albumin levels and the measured parameters as outlined.

TABLE II. LEUCOCYTE NUMBER AND FUNCTION

	Leucocyte count		Cells with NBT (%)		Cells with NBT (%) (with latex)	
	Mean	± SD	Mean	± SD	Mean	± SD
Group I (control I)	8 848	3 326	9,36	1,75	18,14	3,56
Group II (PEM)	9 016	2 806	17,62	9,28	29,22	10,48
Group III (PEM + infection)	8 292	2 708	21,89	11,08	29,18	12,49

TABLE IV. CORRELATION COEFFICIENTS (PEARSONS). LEUCOCYTE NUMBER AND FUNCTION AGAINST TOTAL PROTEIN AND SERUM ALBUMIN LEVELS

	Total protein	Albumin
Group I (control)*		
Leucocyte	0,2784	0,0155
% cells with NBT	0,0752	0,3584
% cells with NBT + latex	0,1677	0,3155
Group II (PEM)*		
Leucocyte	0,2135	0,1199
% cells with NBT	−0,2930	−0,3600
% cells with NBT + latex	−0,3110	−0,3810
Group III (PEM + inf.)*		
Leucocyte	0,3750	0,5150*
% cells with NBT	−0,1095	−0,1665
% cells with NBT + latex	−0,0389	−0,1181

* >0,391 = $P<0,05$.

DISCUSSION

Many of the mechanisms that are possibly implicated, and which result in the increased susceptibility to infection in the malnourished infant, still remain controversial.

Fernandez,[7] and later, Pretorius and De Villiers,[8] reported no difference in serum antibody levels, or antibody response to *Salmonella* antigens, when malnourished infants were compared with those who were well nourished. Many workers,[6,9,12] however, dispute these data, claiming that these infants had received vigorous dietary therapy and were perhaps no longer sufficiently lacking in amino acids to depress antibody response.

Data on type IV Gell and Coombs' cell-mediated response (delayed hypersensitivity) found in malnutrition are also conflicting. Animal experiments have suggested increased, unchanged, or decreased responses,[13] while studies in man have shown a reduced rate of transformation of phytohaemagglutinin-stimulated lymphocytes,[14] anergy to tuberculin[15,16] even following BCG vaccination,[17] and deficient rejection of homologous skin grafts.[18]

Information concerning the number and activity of the leucocytes found in malnourished patients is also somewhat variable. Trowell *et al.*[19] and Behar *et al.*[20] found an absolute or relative deficiency, or both, of leucocytic response in severe kwashiorkor in man, which supported the findings from animal experiments reported by Asirvadham[21] and Guggenheim and Buechler.[22] Seth and Chandra[23] investigated opsonic activity, phagocytosis and bacteria-killing capacity of polymorphs in undernourished patients, and although they found an increase in opsonic

TABLE III. COMPARISON OF THE MEANS FOR LEUCOCYTE NUMBER AND FUNCTION IN THE THREE GROUPS STUDIED

	Cells with NBT		Cells with NBT + latex		Leucocytes	
Group I (control) / Group II (PEM)	$P<0,001$	$P<0,001$	$P<0,001$	$P<0,001$	NS	NS
Group II (PEM) / Group III (PEM + infection)	NS		NS		NS	

activity, they also demonstrated a significant decrease in bacteria-killing capability by the polymorphs. Most of the patients investigated were, however, apparently anaemic, as were the patients reported by Shousha and Kamel,[24] who also found a decreased number of formazan-positive cells in kwashiorkor patients' neutrophils when stained with nitro-blue tetrazolium. No haemoglobin levels are available for those malnourished infants, reported by Kendall and Nolan,[25] who were found to have a decreased NBT response. Iron deficiency apparently adversely affects intracellular bacterial killing and formazan formation by decreasing the activity of myeloperoxidase.[26,27] Studies done on the neutrophils of kwashiorkor patients where this factor has not been taken into account, may be invalid. Arbeter *et al.*[26] could find no deficiency in intracellular leucocyte function, nor could they demonstrate a correlation between degrees of protein deficiency and leucocyte function. The present study supports their data, NBT cells being increased in the malnourished infants compared with the normal group. No correlation was found between total leucocyte count/cells containing NBT (with and without latex stimulation) and serum protein or serum albumin in any of the groups, nor could any difference be demonstrated between those malnourished infants with or without infection.

It appears from the data presented here that the primarily protein-deficient patients' leucocytes function normally as long as gross iron-deficiency anaemia is not present, and that malnourished patients, whether they clinically appear to be infected or not, are all infected.

Finally, as leucocyte numbers were similar in both the normal and malnourished groups, a decreased leucocyte response, in terms of numbers, is obviously present in protein energy malnutrition.

We wish to thank Dr W. Castle, Department of Social Medicine, University of Rhodesia, for statistical assistance; and Dr T. Stamps, City Medical Officer of Health, Salisbury, for allowing us access to his patients.

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