

Nitro blue tetrazolium test in children with kwashiorkor with a comment on the use of latex particles in the test

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SYNOPSIS The nitro blue tetrazolium test was applied on neutrophils from children with kwashiorkor and from control children. The percentage of formazan cells formed in the kwashiorkor patients was significantly less than in the control group in spite of the presence of associated infections in the former. A direct correlation exists between the percentage of formazan cell formation and total serum proteins, albumin, and haemoglobin. These findings may reflect a decreased bactericidal activity of neutrophils in kwashiorkor. The use of latex particles is an unnecessary technical refinement of the test.

Children with kwashiorkor almost invariably suffer from concomitant infections (Trowell, 1949; Williams, 1953; Scrimshaw, Wilson, and Bressani, 1960; Phillips and Wharton, 1968) for which several factors have been blamed. These include decreased antibody production (Brown and Katz, 1965; Scrimshaw, Taylor and Gordon, 1968) and reduced levels of immunoglobulins (Brown and Katz, 1965; Aref, Badr El Din, Hassan, and Araby, 1970), diminished tissue resistance and lowered phagocytic activity of the polymorphonuclear neutrophil leucocytes (Scrimshaw *et al.*, 1959 and 1968).

We have applied the nitro blue tetrazolium test for the study of the integrity of the polymorphonuclear neutrophil leucocytes in children with kwashiorkor as an index of their defence mechanism. This test was introduced by Baehner and Nathan in 1966 for the study of the polymorphonuclear neutrophil leucocytes from children with chronic granulomatous disease who die early in life from repeated infections. In this disease, the capacity of polymorphonuclear neutrophil leucocytes to reduce the nitro blue tetrazolium salt (Baehner and Nathan, 1966 and 1967) and to form formazan cells (Gifford and Malawista, 1970) was found to be greatly diminished.

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Moreover, these leucocytes were incapable of killing certain bacteria (Holmes, Quie, Windhorst, and Good, 1966; Quie, White, Holmes, and Good, 1967) and a correlation was drawn between the power of the cells to reduce nitro blue tetrazolium and their bactericidal activity (Windhorst, Holmes, and Good, 1967).

Cases

Fourteen children with kwashiorkor at the height of the disease, aged between 1 and 3 years, were studied. All of them had diarrhoea and some had also other infections, eg, bronchitis, bronchopneumonia, otitis media, and skin infections. Thirteen well nourished children, free from infections and of the same age group, were studied for comparison.

Methods

The following laboratory determinations were carried out: total serum proteins and albumin, haemoglobin and haematocrit values, and total leucocyte counts. The nitro blue tetrazolium test was done by the method of Gifford and Malawista (1970) for all the cases without incubation with latex particles and for some of them (11 kwashiorkor and 12 control) with such incubation.

Results

Table I shows the clinical and laboratory data of the individuals studied. The mean weights of the kwashiorkor and control children compared to the Harvard

	Case No.	Age (mth)	Percentage of Weight to Normal	Total Serum Proteins (g/100 ml)	Serum Albumin (g/100 ml)	Haemoglobin (g/100 ml)	Haematocrit (%)	Total Leucocyte Count (cells/c mm)
Kwashiorkor	14	24 ± 7	65 ± 13	4.6 ± 1.1	2.0 ± 0.6	7.2 ± 1.2	23.1 ± 5.0	11746 ± 8 200
Control	13	27 ± 9	93 ± 11	7.1 ± 0.5	3.8 ± 0.5	11.2 ± 0.7	32.5 ± 2.4	11965 ± 3 412

Table I Clinical and laboratory data of kwashiorkor and control children (mean ± SD)

standard (Nelson, 1960) were 65 and 93% respectively. The kwashiorkor patients manifested various degrees of hypoproteinaemia, hypoalbuminaemia, and anaemia. Their mean total leucocyte count was within the normal range.

Table II gives the results of the nitro blue tetrazolium test. Particulars of these data are illustrated

		Formazan Cells (%)			
		Without Latex		With Latex	
	No. of Cases	Average ± SD	No. of Cases	Average ± SD	
Kwashiorkor	14	48.6 ± 20.4	11	58.6 ± 20.0	
Control	13	88.9 ± 11.7	12	96.7 ± 3.4	

Table II Results of nitro blue tetrazolium test on kwashiorkor and control children P^1

¹Significance of difference between the mean results for kwashiorkor and control children is <0.001 in both tests.

in Figure 1. Without latex 88.9% of polymorphonuclear neutrophil leucocytes from the control group and 48.6% from children with kwashiorkor were transformed into formazan cells. These are highly significant different results ($P < 0.001$). Upon adding latex particles to the test media, these percentages increased to 96.7% and 58.6% respectively ($P < 0.001$ with a very high direct correlation between the two procedures ($r = 0.95$)).

A direct correlation exists between the percentage of formazan cell formation and the amount of total serum proteins ($r = 0.71$ when the test was carried out without latex and 0.69 when done with it), albumin ($r = 0.78$ and 0.76 respectively), and haemoglobin ($r = 0.67$ and 0.65). In all cases P was < 0.001. All the correlations are thus higher when the test was done without latex and these are illustrated in Figure 2. There is no correlation between the total leucocyte counts and formazan cell formation.

Discussion

The percentage of formazan cell transformation in our laboratory is higher than that of Gifford and Malawista (1970) (88.9-96.7 versus 40%) for the

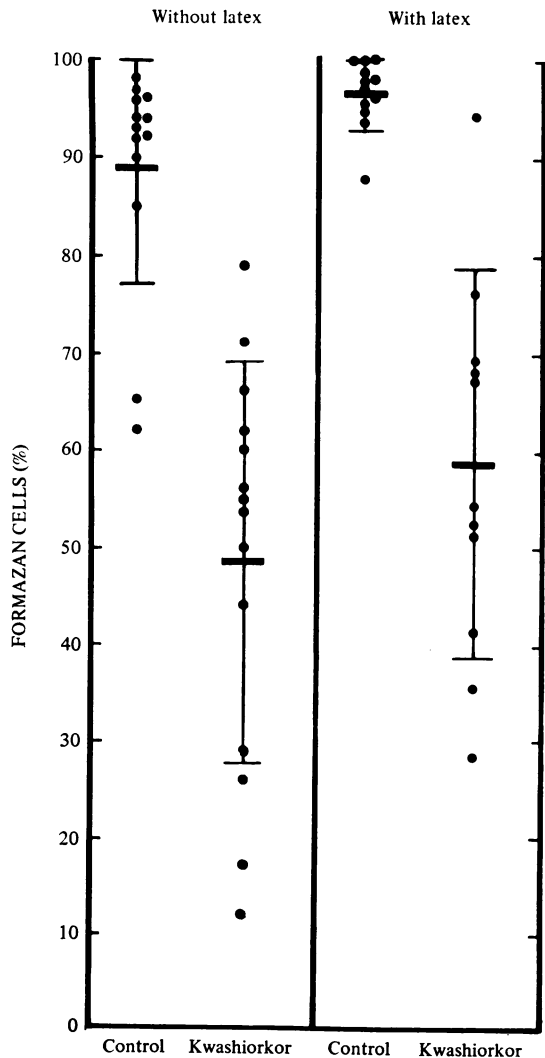


Fig. 1 Formazan cell formation in neutrophils of kwashiorkor and control children in the test as applied without and with latex particle incubation.

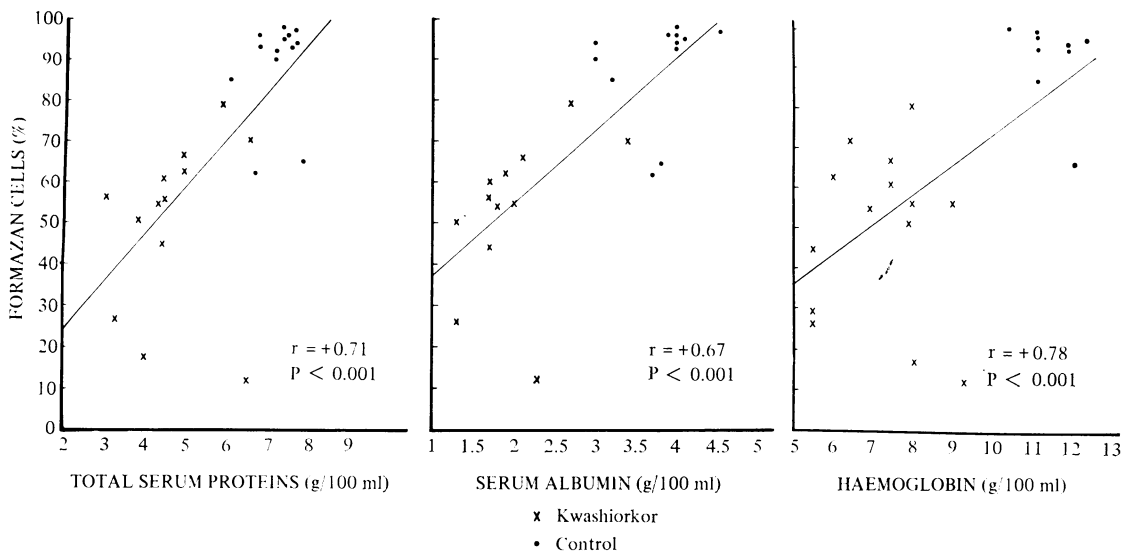


Fig. 2 Correlations between formazan cell formation and total serum proteins, albumin, and haemoglobin by neutrophils of kwashiorkor and control children.

normal control children. Our value is comparable, however, to that of Mandell and Hook (1969).

The stimulating effect of phagocytosis in our study, using latex particles, conforms with previous reported results (Baehner and Nathan, 1967 and 1968). However, as others have pointed (Gifford and Malawista, 1970) this effect seems an unnecessary technical refinement in view of the high correlation between the two procedures.

The reduction of nitro blue tetrazolium by normal polymorphonuclear neutrophil leucocytes depends upon the adequate activation of an enzyme in the phagocytic vacuole (Nathan, Baehner, and Weaver, 1969). The enzyme that is directly or indirectly responsible is thought to be reduced nicotinamide adenine dinucleotide (NADH) oxidase (Baehner and Nathan, 1967; Nathan *et al.*, 1969) which plays a key role in the hexose monophosphate pathway from which the polymorphonuclear neutrophil leucocytes obtain the necessary energy for their bactericidal capacity (Selvaraj and Sbarra, 1966). The activity of that enzyme system and/or its release is thus significantly reduced in kwashiorkor as shown by the decreased ability of the cells in this condition to form formazan cells in spite of associated infections known to enhance this phenomenon (Park, Fikrig, and Smithwick, 1968). These findings indicate reduced bactericidal activity of polymorphonuclear neutrophil leucocytes in kwashiorkor.

The influence of malnutrition upon results of the nitro blue tetrazolium test in kwashiorkor is sug-

gested by the direct relationship between the ability of polymorphonuclear neutrophil leucocytes to form formazan cells and upon total serum proteins, albumin, and haemoglobin levels. The use of this simple test may find its wide application in the assessment of the cells' defence mechanism in those areas where malnutrition is prevalent in its different forms. It is possible that, in the future, the use of leucocyte transfusions could be gratifying whenever deemed advisable in these cases.

The opinions and assertions contained herein are the personal ones of the authors and are not to be construed as official or reflecting the views of the Navy Department, the naval service at large, or the Egyptian Ministry of Health.

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Addendum

The nitro blue tetrazolium test without latex incubation was done on neutrophils from eight children with marasmus. At a mean percentage of 62.3 ± 22.9 ($P < 0.02$), the formazan cell formation in this condition falls midway between normal children and those with kwashiorkor.