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The effects of kwashiorkor serum on lymphocyte transformation *in vitro*

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SUMMARY

The serum from twelve children with kwashiorkor was deficient in its ability to support lymphocyte transformation *in vitro*, whereas lymphocytes from these children responded to phytohaemagglutinin and allogeneic lymphocytes in a relatively normal manner when cultured in normal serum. This serum abnormality improved with therapy and could not be clearly correlated with the degree of malnutrition, the presence or absence of infection or other laboratory manifestations of kwashiorkor. These observations indicate that defective cellular immune reactions in kwashiorkor may be symptomatic of a lack of some humoral factor and do not necessarily reflect an intrinsic cellular defect.

INTRODUCTION

There is considerable evidence, both in the published literature and in common clinical experience, to indicate that infections and their complications occur more frequently and have more serious consequences in children suffering from protein energy malnutrition (PEM) (Trowell, Davies & Dean, 1954; Wittman *et al.*, 1967; Scrimshaw, Taylor & Gordon, 1968) and from kwashiorkor (Pretorius, Davel & Coetzee 1956; Smythe, 1958; Philips & Wharton, 1968; Moorehead *et al.*, 1974).

This increased susceptibility to infection in the malnourished child is generally attributed to the deleterious effects of nutritional deprivation upon the immune mechanisms that serve to prevent or limit bacterial, viral and fungal invasion *in vivo*.

In this paper we draw attention to the fact that the serum from children with kwashiorkor is deficient in its ability to support lymphocyte transformation *in vitro*, whereas lymphocytes from such children respond to mitogenic stimulation in a relatively normal manner when cultured in normal serum. Our observations indicate that defective cellular immune reactions in kwashiorkor may be symptomatic of a lack of some humoral factor and do not necessarily reflect an intrinsic cellular defect.

MATERIALS AND METHODS

Cells and serum. Cells and serum used in the lymphocyte transformation studies were obtained from three sources: (a) children with kwashiorkor (patient cells and serum); (b) healthy children attending a 'well baby' clinic who were matched for sex, age and race with the kwashiorkor patients (control cells and serum); and (c) healthy, adult laboratory personnel or blood donors on the panel of the Western Province Blood Transfusion Service (reference cells and serum). In each individual lymphocyte transformation assay, in which the responses of control or patient cells were studied, reference cells were cultured in parallel to provide a 'normal' reference for comparison.

Lymphocytes were isolated from freshly-drawn, defibrinated, venous blood by the Ficoll–Isopaque gradient technique of Böyum (1974) and suspended in Eagle's minimal essential medium (MEM) (Gibco, U.S.A.) buffered to pH 7·3 with 0·025 M Tris buffer containing 0·02 M L-glutamine and penicillin (100 u) and streptomycin (100 μg) (T–MEM).

Patient and control sera were obtained by allowing venous blood to clot in glass test tubes for 1–2 hr at room temperature.

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The sera were collected by centrifugation, heat-inactivated (56°C for 30 min), sterilized by membrane filtration (0·45 μm filter) and used immediately or stored, in individual aliquots, at −70°C. Reference serum was obtained by pooling 250 ml serum samples from each of four healthy male blood donors of blood group AB, Rh-positive. This serum was heat-inactivated, sterilized and stored in aliquots at −70°C. Under these conditions it retained its original ability to support lymphocyte transformation for at least 1 year. Aliquots were thawed as required; unused serum for each aliquot was discarded.

Informed consent for the studies was obtained from the parents of all patients and control children and the study was performed according to a protocol approved by the Ethical Review Committee of the University of Cape Town.

Patients. Studies were performed on twelve children receiving treatment in the Red Cross War Memorial Children's Hospital. Eleven children had kwashiorkor and one child marasmic kwashiorkor. The nutritional classification was based on the expected weight for age (FAO/WHO Expert Committee on Nutrition, 1971) using Harvard standards (Vaughan, 1975). Children with tuberculosis, measles, severe pulmonary infection or diarrhoeal disease were excluded from the study.

All of the children received parenteral antibiotics (kanamycin sulphate 10 mg/day/kg in two divided doses and procaine penicillin 25,000 u/kg/day for 1 week), oral potassium chloride (3 meq/kg/day for 10 days) and a multivitamin syrup. Supplementary folic acid and ferrous sulphate were commenced in the second week of treatment. Protein and calories were added to the diet gradually and, since lactose intolerance is common in this disease, a casilan cream formula was used initially (Bowie, Barbezat & Hansen, 1967). By 10 days the majority of the children were receiving a full diet. Routine investigations included full blood count, chest X-ray, measurement of serum proteins, urea and electrolytes, and cultures of urine, stool, throat swab and skin lesions. Blood for the initial studies was taken by venipuncture before 9.00 a.m. on the day following admission and before protein re-feeding had commenced.

Laboratory and other patient data relevant to this study are summarized in Table 1.

Lymphocyte transformation. The lymphocyte culture techniques used were based on the method described by du Bois *et al.* (1973). All technical procedures, reagents, tissue culture ware, incubation conditions and the experimental protocol were meticulously standardized in order to avoid variations that any of these items may cause (Schellekens & Eijsvoogel, 1968; Ling & Kay, 1975).

Lymphocytes were cultured in triplicate at 37°C in tightly stoppered 11 × 70 mm polystyrene tubes (Nunc; 1090 Roskilde, Denmark). Each culture contained 3×10^5 lymphocytes suspended in 1 ml of T–MEM supplemented with 20% serum.

Phytohaemagglutinin (PHA) induced blastogenesis was achieved by the addition of 1 μg of purified PHA (Wellcome, Beckenham, England) in 50 μl of T–MEM to each culture at the start of incubation. One-way mixed lymphocyte reactions were assayed by culturing $1{\cdot}5 \times 10^5$ responder cells with $1{\cdot}5 \times 10^5$ allogeneic cells that had been preincubated at 37°C for 30 min by mixing an equal volume of defibrinated blood with T–MEM containing 0·05 mg/ml of mitomycin C (A. Christiaens N.V., Brussels) prior to separation.

The lymphocyte cultures were incubated at 37°C for a total of 3 days in the case of PHA-stimulated cultures and for 6 days in the case of mixed lymphocyte cultures. 24 hr before harvesting, 0·075 μCi of [2-^{14}C]thymidine (specific activity 60 mCi/mmol; Amersham, England) was added to each tube.

Lymphocytes were harvested by vacuum filtration on a Millipore filtration unit fitted with a glass fibre pre-filter. The residual contents of the culture tubes were rinsed on to the filters with two 3 ml washes of 0·9% NaCl. Free radioactivity was effectively removed from the filters with two 10 ml washes with distilled water. The damp filters, with the washed lymphocytes, were placed in glass liquid scintillation vials and dried for 1 hr at 60°C. 5 ml of liquid scintillation fluid (Instagel, Packard, U.S.A.) were added to each vial and the samples were counted in a Packard Tri-carb scintillation spectrophotometer. Counts per minute (ct/min) were corrected to disintegrations per minute (d/min) by constructing a quench curve from the AES ratio and percentage efficiency of standard quenched samples.

Total serum protein was measured by the biuret method (Henry, Sobel & Berkman, 1957) and the albumin and globulin fractions by quantitative densitometry of cellulose acetate microelectrophoresis strips. Serum IgG, IgM and IgA concentrations were measured by single radial immunodiffusion in Behringwerke Tripartigen plates. Standard serum samples for these determinations were calibrated against reference serums 67/97 obtained from the WHO International Reference Centre for Immunoglobulins.

Serum C3 concentrations were measured by radial immunodiffusion, using commercial plates and standards (Behringwerke, Germany). Serum IgE was measured using the double antibody radioactive radial immunodiffusion technique described by Orren & Dowdle (1975).

Statistical analysis. The Student's *t*-test was used to estimate the levels of significance for differences between the means of data summarized in Table 1.

The Friedman non-parametric two-way analysis of variance was used to analyse the data summarized in Fig. 1.

Wilcoxon's matched-pairs signed-ranks test was used to analyse the data summarized in Table 2.

Differences between data sets were regarded as significant if the null hypothesis could be rejected at the 1% level or less.

RESULTS

All of the children recovered and it was possible to repeat most of the investigations on nine children after 2 weeks, on six children after 4 weeks and on five children after 6 weeks. Pathogenic organisms

TABLE 1. Nutritional, clinical and biochemical results in ten control children and twelve children with kwashiorkor tested on admission to hospital and at various time intervals after the institution of nutritional therapy

Characteristic studied	Control children	Kwashiorkor patients tested on admission and at various intervals during recovery			
		Admission	2 weeks	4 weeks	6 weeks
No. of subjects tested	10	12	9	6	5
Age (months)	20·4 (11–44)*	25·1 (10–48)	28 (18–48)	29 (18–48)	23 (18–30)
Sex ratio F:M	6:4	8:4	5:4	2:4	4:1
Percentage expected weight for age	103·4 (85–117)	70·4 (53–80)	74·4 (57–84)	78·7 (62–88)	77·4 (70–88)
Total protein (g/100 ml)	6·65 (5·87–7·12)	4·75 (3·68–5·14)	7·32 (6·84–8·15)	7·61 (7·22–8·09)	7·71 (7·05–8·44)
Albumin (g/100 ml)	3·76 (3·26–4·21)	1·86 (1·3–2·31)	3·36 (2·92–3·73)	3·71 (3·53–3·97)	3·84 (3·45–4·08)
α1 globulin (g/100 ml)	0·24 (0·20–0·28)	0·27 (0·2–0·34)	0·28 (0·21–0·32)	0·26 (0·22–0·29)	0·25 (0·20–0·28)
α2 globulin (g/100 ml)	0·80 (0·68–0·88)	0·69 (0·4–0·92)	1·05 (0·76–1·53)	0·88 (0·63–1·09)	0·91 (0·63–1·17)
β globulin (g/100 ml)	0·85 (0·71–1·04)	0·62 (0·37–0·85)	1·15 (1·07–1·19)	1·09 (0·92–1·27)	0·94 (0·82–1·11)
γ globulin (g/100 ml)	1·00 (0·61–1·39)	1·30 (0·87–1·63)	1·47 (1·13–1·91)	1·67 (1·41–2·27)	1·78 (1·43–2·41)
IgG (mg/100 ml)	937 (474–1446)	1519 (474–2180)	1690 (1150–2260)	1628 (1190–2020)	1779 (1240–2483)
IgM (mg/100 ml)	122 (66–200)	232 (122–380)	233 (95–320)	241 (158–336)	205 (146–297)
IgA (mg/100 ml)	81 (28–135)	182 (83–302)	195 (105–306)	181 (22–285)	114 (52–204)
IgE (u/ml)	70 (10–130)	697 (26–2810)			
C3 (mg/100 ml)	155 (106–236)	95 (43–179)	197 (160–224)	170 (119–202)	169 (149–202)

* Mean values; range in parentheses throughout.

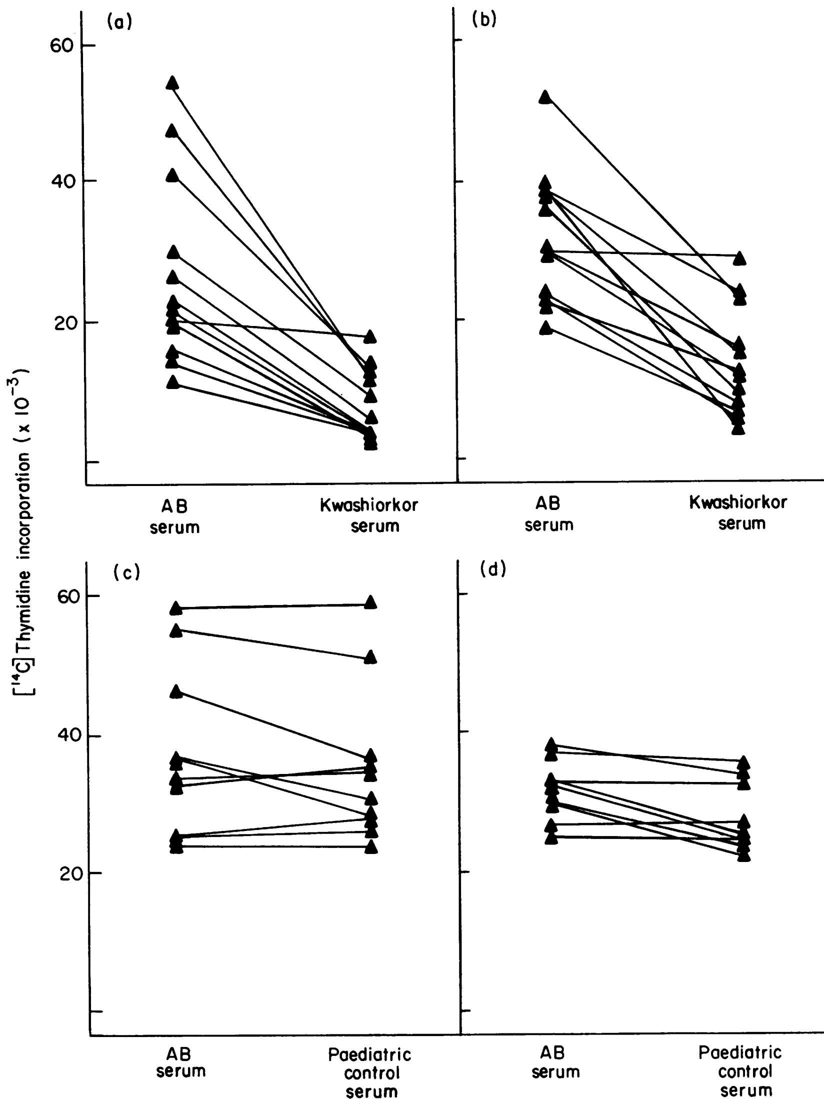

FIG. 1. PHA-induced incorporation of [^{14}C]thymidine into lymphocytes cultured in media supplemented with the sera indicated at the bottom of each panel. Each point represents the mean result obtained from triplicate cultures. Pairs of points connected by lines represent results obtained when the same lymphocytes were cultured in two different sera in the same experiment. Peripheral blood lymphocytes were isolated from the following subjects: (a) patients with kwashiorkor; (c) paediatric control subjects; (b) and (d) healthy adults (reference cells). The suppressive effect of the kwashiorkor serum on autologous and reference cells was significant ($P < 0{\cdot}001$) by Friedman's non-parametric two-way analysis of variance.

were isolated from stool specimens in eight children, and from throat swabs or skin swabs in four children. Three children had X-ray evidence of minor pneumonic changes. One child developed measles 13 days after admission. Relevant clinical and laboratory details of the twelve patients with kwashiorkor and the ten normal children are summarized in Table 1. No significant differences in age or in sex ratios between the two groups were present. Serum protein analyses showed significantly lower values for total protein, albumin, $\alpha 2$ globulin, β globulin and C3 in the kwashiorkor group ($P < 0{\cdot}001$). Despite the wide ranges of the values observed for individual immunoglobulin classes, IgM, IgA and IgE concentrations were significantly higher in the patient sera ($P < 0{\cdot}001$).

TABLE 2. Results of eight one-way mixed lymphocyte cultures of patient and reference lymphocytes in patient and AB serum. Results given as means of d/min ^{14}C incorporated into $1{\cdot}5\times 10^{5}$ responder cells

Responder lymphocytes: Stimulating lymphocytes: (mitomycin-treated)	Kwashiorkor Control	Control Kwashiorkor	P*
AB serum	6526 (1628)†	5498 (1632)	n.s.
Kwashiorkor serum	1686 (718)	1234 (550)	n.s.
P*	< 0·01	< 0·01	

* Wilcoxon matched-pairs signed-ranks test.
† s.e.m. in parentheses.

By 2 weeks the total serum protein, albumin and globulin fractions were comparable to those of the normal children. This improvement was consolidated during the next 4 weeks. Levels of C3 surpassed normal values at 2 weeks and fell back again after the next 4 weeks. There was little change in the immunoglobulin levels over the 6 week period, except that serum IgG values rose to even higher levels.

Mitogen-induced lymphocyte transformation is a complex process that involves both viable, mitogen-responsive cells and serum components that are required to support cellular DNA synthesis and proliferation. In order to distinguish between the effects of malnutrition upon the cellular and the serum components of the *in vitro* lymphocyte response, twelve 'cross-over' experiments were performed, in which lymphocytes from kwashiorkor patients or from healthy adult blood donors (reference cells) were incubated in patient serum and in reference AB serum. The results of these experiments are presented graphically in Fig. 1a and b.

As is evident from these data, the PHA-induced incorporation of [^{14}C]thymidine into both reference and patient lymphocytes was consistently and significantly ($P<0{\cdot}001$) lower in the presence of patient serum than in the presence of reference AB serum. No significant intrinsic cellular defect was evident in patient cells, since these showed [^{14}C]thymidine incorporation values comparable to the reference cells when both were incubated in reference AB serum. Mean ^{14}C incorporation values for the various

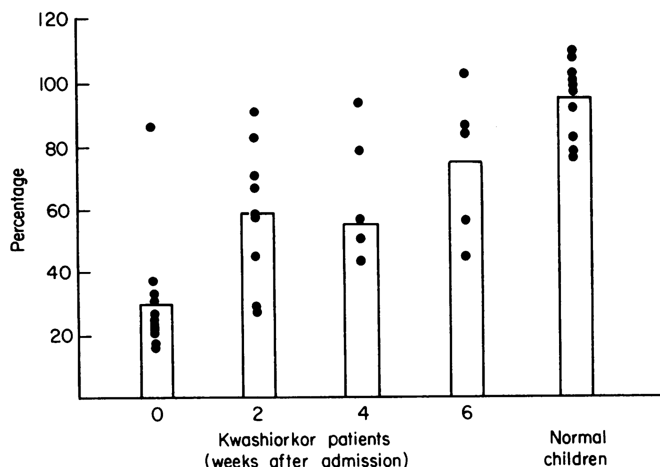

FIG. 2. PHA-induced lymphocyte transformation of lymphocytes obtained from patients with kwashiorkor on admission and at various time intervals after nutritional therapy had been instituted. Values obtained for [^{14}C]thymidine incorporation in the presence of autologous serum are expressed as percentages of values obtained in the presence of normal AB serum. Similar values for ten age-matched well-nourished control children are shown, for comparison, on the right.

TABLE 3. *In vitro* studies of cellular immune function in protein malnutrition

Classification of PEM	Lymphocyte count	Lymphocyte transformation	Active serum	E rosettes	Reference
PEM	14/112 (↓)	(↓)	Autologous	—	Smythe *et al.* (1971)
K	—	12/16 (↓)	Albumin	—	Geefhuysen *et al.* (1971)
K	—	(↓)	FCS	—	Sellmeyer *et al.* (1972)
PEM	15/90 (↓)	(↓)	?	—	Chandra (1972)
PEM	—	(↓)	Autologous	—	Grace *et al.* (1972)
PEM	—	6/16 (↓)	AB	—	Burgess *et al.* (1974)
PEM	—	(↓)	Autologous	—	Burgess *et al.* (1974)
K plus M	—	N	FCS	—	Moore *et al.* (1974)
PEM	—	4/14 (↓)	AB	—	Coovadia *et al.* (1974)
PEM	—	(↓)	AB	(↓)	Chandra (1974)
PEM	N	N	?	(↓)	Ferguson *et al.* (1974)
K	N	(↓)	?	(↓)	Bhaskaram & Reddy (1974)
K	7/20 (↓)	—	—	—	Rosen *et al.* (1975)
M	—	N	?	—	Schlesinger & Stekel (1974)
PEM	—	—	—	(↓)	Bang *et al.* (1975)
K plus M	3/33 (↓)	5/27 (↓)	?	—	Neumann *et al.* (1975)
PEM	—	(↓)	Autologous	—	Heyworth *et al.* (1975)
K	N	(↓)	FCS	(↓)	Schopfer & Douglas (1976)

PEM, Protein energy malnutrition (unspecified); K, kwashiorkor; Autologous, autologous serum; M, marasmus; AB, human blood group AB serum; (↓), decreased; FCS, foetal calf serum; N, normal; ? serum source not specified; —, not done.

cell–serum combinations were as follows: patient cells in AB serum, 27,335 d/min; patient cells in autologous patient serum, 7844 d/min; reference cells in AB serum, 32,000 d/min; reference cells in patient serum, 13,727 d/min.

To examine the possibility that these results might simply reflect differences between adult and infant sera in their ability to support lymphocyte transformation, cells and sera from healthy paediatric control subjects were compared with the reference lymphocytes and AB serum in a further ten similarly designed experiments. The results of this series (Figs. 1c and d) showed that the [^{14}C]thymidine incorporation into control cells was more variable and generally greater than it was in the case of adult reference lymphocytes. No consistent or significant serum effect was evident, either in the case of control or reference cells. Mean [^{14}C]thymidine incorporation values in this series were as follows: control cells in AB serum, 36,522 d/min; control cells in autologous control serum, 35,286 d/min reference cells in AB serum, 31,718 d/min; reference cells in control serum, 27,426 d/min.

Finally, a comparison of the results obtained in the two series of experiments indicates that mean ^{14}C incorporation rates for patient cells and control cells were similar when incubated in the reference AB serum, (Fig. 1a and c), further suggesting that lymphocytes from patients with kwashiorkor are capable of responding normally to PHA, provided they are cultured in the presence of normal serum.

The results obtained with the mixed lymphocyte cultures showed an essentially similar picture (Table 2). Patient and reference lymphocytes both responded to and stimulated allogeneic cells in a quantitatively similar way when cultured in healthy AB serum. Responses of both patient and reference cells were, however, significantly diminished when incubated in patient serum.

Clinical improvement of the patients with therapy was accompanied by an increase in the ability of their sera to support PHA-induced lymphocyte transformation. In Fig. 2 the lymphocyte responses in autologous patient sera are expressed as a percentage of the response in the AB serum and plotted as a function of time following the institution of therapy. By 2 weeks there was a significant increase in the mean value; by 6 weeks all of the five patients studied showed considerable improvement in the serum effect, with three of them having values within the control range.

We were unable to discern any clearly defined or consistent relationship between the magnitude of the serum defect and the severity of the disease as judged by clinical or laboratory criteria. Similarly, the extent to which patient sera were inadequate for sustaining lymphocyte transformation was not obviously related to the presence or absence of overt infection, the serum C3 concentration or the serum concentration of any particular class of immunoglobulin.

DISCUSSION

Circumstantial clinical and laboratory evidence would suggest that the predominant immunopathological consequences of malnutrition are borne by cell-mediated immune functions. Atrophy of the thymus gland and other lymphoid organs in kwashiorkor has been well-documented (Vint, 1937; Watts, 1969; Mugerwa, 1971; Smythe *et al.*, 1971; Purtillo & Connor, 1975); delayed hypersensitivity reactions to common antigens are absent or blunted (Harland & Brown, 1965; Lloyd, 1968; Chandra, 1972; Abassy *et al.*, 1974; Geefhuysen *et al.*, 1971; Neumann *et al.*, 1975), and DNCB sensitization and recall is impaired (Smythe *et al.*, 1971; Chandra, 1972; Edelman *et al.*, 1973; Coovadia *et al.*, 1974; Bang *et al.*, 1975).

Furthermore, total serum Ig levels are usually normal or raised in PEM (Keet & Thom, 1969; Watson & Freeseman, 1970; McFarlane *et al.*, 1970; El-Gholmy *et al.*, 1970; Geefyhuysen *et al.*, 1971; Rosen, Geefhuysen & Ipp, 1971; Smythe *et al.*, 1971; Alvardo & Luthringer, 1971; Chandra, 1972; Coovadia *et al.*, 1974; Neumann *et al.*, 1975). Studies of humoral responses have given inconsistent results (Pretorius & De Villiers, 1962; Reddy & Srikantia, 1964; Brown & Katz, 1965; Chandra, 1972, 1975a; El-Molla *et al.*, 1973; Coovadia *et al.*, 1974; Neumann *et al.*, 1975; Suskind *et al.*, 1976). These inconsistencies may have been due to the fact that in most cases the children were given a high protein diet following immunization and prior to testing (Scrimshaw *et al.*, 1968).

Defects in non-specific immunity can also be implicated in the increased susceptibility of the malnourished child to infection. Phagocytic cell function in PEM has been reported to be both normal (Tejada *et al.*, 1964; Arbeter *et al.*, 1971) and abnormal (Seth & Chandra, 1972; Selveraj & Bhat, 1972a, b; Douglas & Schopfer, 1974). Rosen *et al.* (1975) showed depressed neutrophil bactericidal and chemotactic activity in infected kwashiorkor and control children when compared with uninfected kwashiorkor and control children.

Low levels of serum complement components have been described in PEM (Smythe *et al.*, 1971; Chandra, 1972; Sirisinha *et al.*, 1973; Coovadia *et al.*, 1974; Neumann *et al.*, 1975; Chandra, 1975b). The levels rise rapidly to normal with nutritional recovery and the abnormalities are felt to be due to decreased synthesis rather than increased consumption (Sirisinha *et al.*, 1973).

One might suspect, therefore, that specific immunodeficiency of malnutrition represents the effects of a prolonged dietary deficiency of some factor(s) essential for the normal development and function of thymus-derived lymphocytes, since it is these cells that are generally accepted as the mediators of specific cellular immunity.

Although most published studies of lymphocyte transformation in protein energy malnutrition have reported diminished cellular responses to mitogenic stimuli (Table 3), the importance of the source of serum used to supplement the lymphocyte cultures has not been emphasized. This factor, together with technical differences in assay procedures, variations in the manner in which results have been interpreted and the use of different diagnostic criteria, may have been responsible for some of the discrepancies in the results obtained.

Histological methods were used to assess lymphocyte responses to PHA stimulation in the reports by Smythe *et al.* (1971), Grace, Armstrong & Smythe (1972) and Geefhuysen *et al.* (1971). In all of these studies cultures were established in autologous serum and low levels of transformation were found, although some of the patients studied by both groups had normal responses.

The results have been more conflicting when the incorporation of radioactive thymidine into lymphocyte DNA has been used to quantify the responses to PHA. Sellmeyer *et al.* (1972) and Schopfer & Douglas (1976) found profoundly diminished responses to PHA in lymphocytes from patients with

kwashiorkor. In both studies the lymphocytes were cultured in foetal calf serum (FCS). Moore, Heyworth & Brown (1974), also using FCS, found abnormal results in only two out of sixteen children with severe protein energy malnutrition (three kwashiorkor, six marasmic kwashirokor and seven marasmus). Neumann *et al.* (1975) found low responses in four out of eighteen children with kwashiorkor, but do not mention the type of serum used to supplement their cultures. Chandra (1972, 1974) reported that all of the patients studied by him showed depressed lymphocyte responses to PHA. In the first report the type of serum used in the cultures was not mentioned; in the second study AB serum was used. His patients were classified as having PEM and not all had kwashiorkor. Burgess *et al.* (1974) measured lymphocyte transformation in a group of patients with PEM using both histological and radioactive techniques. They reported a discrepancy in the results obtained with these tests in that, by microscopical evaluation of stimulated cultures, the majority of patients showed a diminished response to PHA, whereas nucleic acid synthesis as measured by the incorporation of [^{3}H]thymidine and [^{3}H]uridine was relatively normal. It should be noted that autologous serum was used to supplement the cultures evaluated histologically, whereas cultures evaluated by isotopic methods were supplemented with AB serum.

Heyworth, Moore & Brown (1975) have reported that heparinized plasma from some children with acute PEM caused marked depression of homologous lymphocyte transformation responses to PHA, that did not appear to be due to plasma cytotoxicity. The most severe depression was observed with plasma from patients with kwashiorkor who subsequently died; the effect of plasma from patients with marasmus was minimal.

In the study that we report it was clearly shown that lymphocyte responses to PHA and allogeneic cells were diminished in cultures supplemented with serum from children with kwashiorkor at the height of the disease. The serum abnormality improved with therapy. Since we have not studied children with PEM without kwashiorkor, we are unable to comment on the findings in marasmus alone.

The nature of the serum abnormality has not been established at this point, although preliminary studies indicate that it reflects a deficiency of an essential serum factor rather than the presence of a cytotoxic factor or an inhibitor. In a subsequent paper (Beatty & Dowdle, in preparation) we shall present the results of our attempts to define the nature of the deficiency. There were certainly no clearly defined correlations with depressed serum albumin, $\alpha 2$ and β globulin and C3 concentration, or with elevated serum immunoglobulin concentrations, to indicate that abnormality of these serum protein components might be responsible.

We have concluded, on the basis of a statistical analysis of PHA-induced mitogenic responses in AB serum, that intrinsic lymphocyte function in kwashiorkor is not abnormal. It should be emphasized, however, that this conclusion may well be a matter of interpretation rather than a matter of fact. If, instead of using grouped statistical methods to analyse the data, one were to define, from the control results, a 'normal' range for thymidine uptake, one might argue that in four cases (Fig. 1a) patient cells showed abnormally low responses in AB serum. Our data might be interpreted, therefore, as indicating that one-third of patients with kwashiorkor showed defective lymphocyte function unrelated to the serum effect. We do not contest this observation, particularly in view of the published reports of thymic depletion (Vint, 1937; Watts, 1969; Mugerwa, 1971; Smythe *et al.*, 1971; Purtillo & Connor, 1975) and diminished numbers of peripheral blood T cells, as measured by sheep erythrocyte rosetting techniques (Chandra, 1974; Ferguson *et al.*, 1974; Bhaskaram & Reddy, 1974; Bang *et al.*, 1975; Schopfer & Douglas, 1976) in kwashiorkor.

Although neither the nature nor the significance of the serum abnormality in kwashiorkor have been established, it may have relevance to the immune function *in vivo* if, as seems probable, effective cellular immune responses and the clonal generation of memory cells require antigen-triggered blastogenesis. The effects of nutritional deprivation may then be mediated by depletion of plasma factors essential for lymphocyte proliferation.

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