# Pattern of Biochemical and Immune Recovery in Protein Calorie Malnutrition

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## SUMMARY

**Thirty-two Black children with protein calorie malnutrition were studied with a view to assessing the duration of derangement of biochemical and immunological factors. It was shown that the majority of biochemical parameters were back to normal within 20 days, while the return to normal of the immunological indices was more protracted. Reference is made to the patients who died, and to the factors contributing to their deaths.**

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The recovery from protein calorie malnutrition (PCM) has been divided into two phases: initiation of recovery and the consolidation phase.[1] The former is the rapid return to normal of some of the clinical and many of the biochemical abnormalities. The latter refers to the period when growth starts once again. Depression of cell-mediated immunity has been shown to occur in PCM,[2] and a study was undertaken to detect when recovery occurs. Serum proteins and related constituents were measured simultaneously in order to detect an association. The effect of measles vaccine on the pattern of recovery was also assessed.

## PATIENTS

Thirty-two Black patients with PCM were taken at random from the children admitted to the Paediatric Unit at King Edward VIII Hospital in Durban. Six died soon after admission, while 2 died in hospital during the period of follow-up. The age range was 8 months to 6 years. They all received routine therapy, which included a high protein diet, vitamins and antibiotics when necessary. Twenty-two of these patients received a one-fifth dose of live, attenuated measles vaccine manufactured by Glaxo Laboratories as Melvelin/L on admission.

Routine investigations (full blood count, chest X-ray film, blood culture, urinalysis, stool microscopy, and tuberculin test) were carried out on admission on all patients.

The following serial investigations were undertaken at 10-day intervals: serum protein, $C_3$ levels and transferrin in all children; delayed hypersensitivity reaction using dinitrochlorobenzene (DNCB) in 23 children; morphology of phytohaemagglutinin (PHA)-stimulated lymphocytes, plasma cortisol (total and percentage unbound), immunoglobulins and C-reactive protein in 12 children; RBC antiglobulins in 8 patients, and tritiated thymidine uptake of PHA-stimulated lymphocytes in 14.

Controls were healthy children, matched for age and race.

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## METHODS

In the determination of serum $C_3$, immunoglobulins (IgG, IgM, IgA) and transferrin, the simplified radial immunodiffusion method of Hyland was employed, using monospecific antisera (heat inactivation or prolonged storage of sera was avoided), cortisol[3] and the morphology of lymphocyte transformation by a method previously described,[2] and DNCB sensitisation as recommended by WHO.[4] (Repeat sensitisation was carried out if no reaction was observed after 2 consecutive challenge doses at 10-day intervals.) Tritiated thymidine uptake of PHA-stimulated lymphocytes was determined by the method described by Burgess *et al.*[5]

## RESULTS

Fig. 1 shows the mean biochemical levels and immune factors on admission and during the recovery phase of children with PCM. Table I expresses these findings as a percentage of children reaching normal values.

**Serum albumin** concentration was low on admission, and rose to 2,8 g/100 ml, which is the critical level for the diagnosis of PCM in our Unit, in 83% of the children by day 10. The mean level of 66 controls (3,7 g/100 ml) was reached in 74% by day 30.

**Serum transferrin** was low in 93% of children on admission, and rose to normal levels by day 20 in 90% (40 controls: 257,7 mg/100 ml ±69,5).

**Serum $C_3$** was reduced in 96% of children on admission, and returned to normal by day 10 in 95% (19 controls: 141,0 mg/100 ml ±21,7).

**Serum $C_4$**: the RBCs of 8 patients were tested for the presence of serum complement components. In 4 cases $C_4$ was detected on admission, disappearing in 10 days only to reappear in 1 patient during an attack of chickenpox.

**Plasma total cortisol** was always raised on admission and reached normal levels by day 30 in 77% of children (10 controls: 10,2 µg/100 ml ±2,3). However, there was considerable fluctuation in individual cases, raised values being concomitant with episodes of infection.

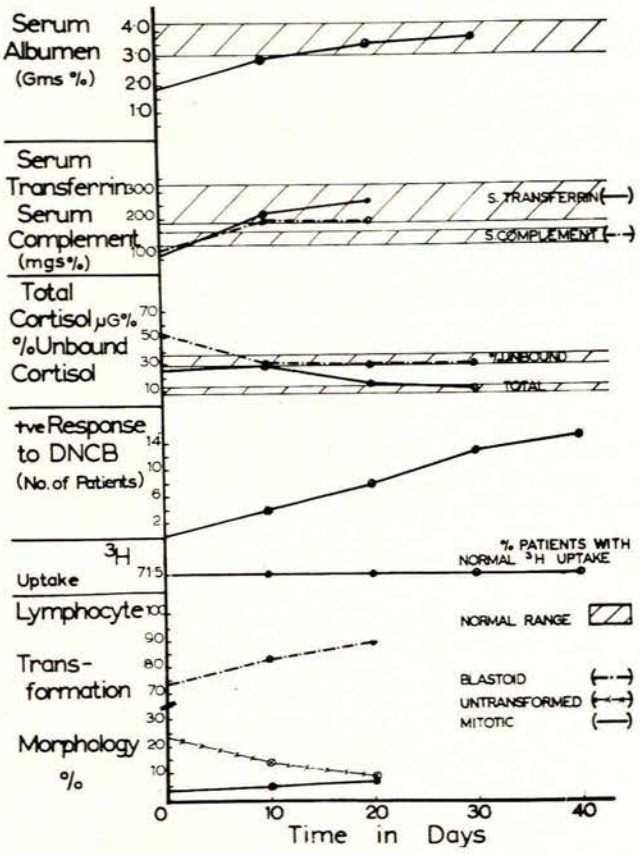


**Fig. 1. Mean biochemical levels and immune factors on admission and during the recovery phase of PCM.**

**Percentage unbound cortisol** was high in all patients on admission and dropped to normal levels by day 10 in 83% (10 controls: 33,0% ±13,8).

**Immunoglobulins**: little derangement was found in the immunoglobulin levels (IgG, IgM, IgA). One patient had significantly low IgG and IgM levels, which returned to normal by days 36 and 20, respectively. IgA levels which were raised in 5 of 8 patients on admission, returned to normal during the period of observation.

**Delayed hypersensitivity reaction by DNCB sensitisation:** 23 patients were initially sensitised with DNCB (Fig. 2) and 19 were followed up beyond 10 days. Four patterns of response could be identified:

(a) a feeble response within the normal period (4 patients);
(b) a delayed response, with a reaction on the second challenge on day 20 (4 patients). This indicates the ability to stimulate memory cells without the concomitant production of sensitised effector cells;
(c) a response after 2 or more sensitisations (8 patients); and
(d) persistent failure to respond to sensitisation up to 60 days (3 patients).

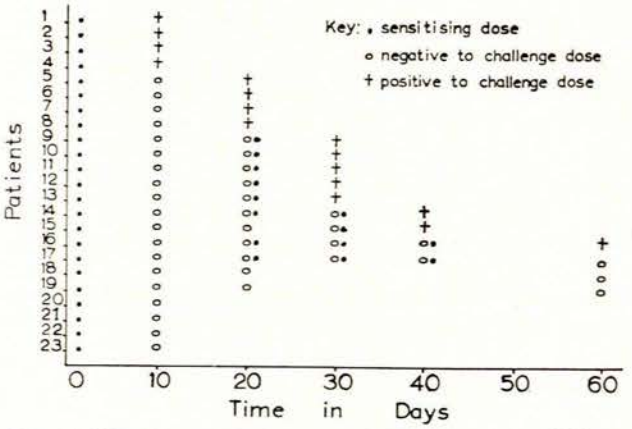


**Fig. 2. DNCB sensitisation on admission and at 10-day intervals in patients with PCM.**

TABLE I. PERCENTAGE OF PATIENTS WITH NORMAL VALUES AT 10-DAY INTERVALS

| | Day 0 | | Day 10 | | Day 20 | | Day 30 | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| Albumin 2,9 g/100 ml | 26 | 0 | 12 | 83 | 13 | 95 | 9 | 100 |
| Albumin 3,7 g/100 ml | 26 | 0 | 24 | 8 | 13 | 60 | 10 | 74 |
| Complement | 30 | 3,3 | 21 | 95 | | | | |
| Transferrin | 31 | 6,5 | 22 | 62,5 | 17 | 90,6 | 4 | 97 |
| Total cortisol | 12 | 0 | 12 | 17 | 11 | 38,5 | 8 | 77 |
| % Unbound cortisol | 12 | 0 | 12 | 83 | 11 | 100 | | |
| +ve reaction to challenge dose of DNCB | 23 | 0 | 23 | 17 | 19 | 42 | 9 | 76 |
| 3H-T uptake by PHA-stimulated lymphocytes | 14 | 71,5 | 14 | 71,5 | 14 | 71,5 | 14 | 71,5 |
| Morphological assessment of PHA-stimulated lymphocytes: | | | | | | | | |
| Untransformed | 10 | 0 | 12 | 16,6 | 6 | 37,5 | 2 | 60 |
| Blastoid | 10 | 0 | 12 | 25 | 6 | 66,6 | 2 | 87,5 |
| Mitotic | 10 | 50 - | 12 | 64 | 6 | 94 | 2 | 88 |

Corrected percentage used: i.e. No. of +ves on previous days added to total number of patients studied on day under consideration.

In order to determine the duration of reactivity to the initial sensitisation with DNCB, 3 patients who had earlier responded to a challenge dose were rechallenged between day 50 and 60. Two of these retained the ability to react, whereas 1 did not. The sensitising effect of DNCB can be lost also in normals by the 50th day.[6]

**Lymphocyte transformation on morphological assessment:** Results showed an impairment in all patients on admission. When compared with controls the dominant effect of PCM was on untransformed ($P<0,01$) and blastoid cells ($P<0,01$), while the percentage of mitotic figures was less affected ($P = 0,15$). By day 30, untransformed cells had reached normal values in 60% of patients, blastoid in 87,5% and mitotic in 80% (9 controls: untransformed 4,54% ±1,36; blastoid 90,92% ±1,47; mitotic 4,55% ±0,98).

**With radio-isotopic evaluation** reduced uptake of tritiated thymidine in PHA-stimulated lymphocytes was observed in 29% of children on admission and persisted for 30 days (10 controls: 414,736 ±88,074).

**TABLE II. SERUM ALBUMIN, SERUM TRANSFERRIN AND CORTISOL LEVELS IN PCM CORRELATED WITH PHYTOHAEMAGGLUTININ TRANSFORMATION OF LYMPHOCYTES MEASURED MORPHOLOGICALLY AND BY TRITIATED THYMIDINE UPTAKE**

| | Correlation coefficient *r* | | | |
|---|---|---|---|---|
| | 3H-T | Untransformed | Blastoid | Mitotic |
| **Serum albumin** | **0,028** | **0,008** | **0,047** | **0,525** |
| **Serum transferrin** | **0,147** | **0,050** | **0,026** | **0,423** |
| **Total cortisol** | — | **0,593** | **0,521** | **0,142** |
| **Unbound cortisol** | — | **0,751** | **0,665** | **0,196** |

Table II shows the correlation between biochemical levels and lymphocyte transformation on admission. Serum albumin and transferrin showed no correlation. Total cortisol showed a fall-off pattern, with its most significant correlation in untransformed cells. The percentage of unbound cortisol showed the highest correlation in untransformed cells and less so in blastoid cells. It must be stressed, however, that there was no correlation with cortisol levels during the period of recovery.

**Blood culture** was positive in 2 of the 24 patients who survived (1 *E. coli* and 1 *Salmonella* species), and in 5 of the 8 patients who died (3 *Pseudomonas*, 1 *E. coli* and 1 *Salmonella* species).

**Postmortem study:** In the patient who died 3 weeks after admission, there was atrophy of the thymus and the thymic-dependent areas in the spleen and peripheral lymph nodes, with barely discernible germinal centres.

## DISCUSSION

Within 20 days of protein refeeding the biochemical parameters were back to normal in the majority of patients. Lymphocyte function, as assessed by the delayed hypersensitivity reaction and morphology of lymphocyte transformation, had recovered by the 30th day.

The remarkable difference of the lymphocyte transformation, when measured by radioactive isotope uptake and when assessed morphologically, was a surprising finding and has been discussed more fully elsewhere.[7] It was thought to be due to the fact that the two investigations assess lymphocyte transformation at different stages of cell division. Large quantities of thymidine are taken up by the cell before there are any morphologically recognisable changes.

Improvement in the protein-related constituents was not paralleled by the recovery of the delayed hypersensitivity reaction, which became positive in only 42% of patients by day 20. There was no correlation between the severity of protein deficiency, as assessed by albumin and transferrin levels on admission, and the duration of depression of cell-mediated immunity as expressed by DNCB sensitisation and lymphocyte transformation.

A gradual improvement in the response to DNCB sensitisation during the period of observation was noted. It is presumably a reflection of the recovery of the thymolymphatic system. A contributing factor may have been the decreasing oedema of the skin and subcutaneous tissue. The latter, however, usually disappears within days after commencement of therapy and, as can be seen from Table 1, on day 20 only 42% of all the patients had a normal response.

The correlation of rate of recovery and the amount of protein in the diet with the rapid return to normal of $C_3$ levels, suggest that the low levels in malnourished children are due to deficient production rather than consumption in an auto-immune process.[8]

Raised cortisol levels in PCM may be a contributory factor in depressing thymolymphatic activity.[9] Cortisol levels returned rapidly to normal, whereas depression of lymphocyte function was more prolonged. This may be partially explained by the postulate that hypercortisolism of short duration may have a prolonged depressant effect on cell-mediated immunity.[10]

The reappearance of serum $C_4$ on the RBCs of 1 patient during an attack of chickenpox suggests that this phenomenon is mediated by infection.[2]

Comparing the patients who died soon after admission with those who survived, one finds some striking differences. Of those who succumbed, all had a strongly positive C-reactive protein (+++ to ++++) with a high incidence of Gram-negative septicaemia and a high number of untransformed lymphocytes. The highest number of untransformed cells was seen in 2 of the patients who died 3 days after admission. Only 2 of the 26 patients who survived had septicaemia. These facts draw attention to the important role of severe infection in the fatal outcome of PCM.

One of the 5 patients with a Gram-negative septicaemia is of particular interest, since after initial improvement, when all parameters had returned to normal except total cortisol and a rising number of untransformed lymphocytes, he died. From the postmortem study, it should be noted that despite the return to normal of many of the

parameters, there was marked depletion of the thymic and lymphoid tissue, but whether this was nutritionally determined or due to the infection is open to speculation.

Measles infection and vaccination are known to depress the cell-mediated immunity.[11-13] The effect of the latter on the rate of immune recovery was assessed by withholding the vaccine from 4 of the patients. As the return to normal of the lymphocyte transformation and delayed hypersensitivity reaction in this group is not more rapid than in the vaccinated group, it is unlikely that the vaccine had a depressing effect. Comparison of the serum transferrin, albumin, serum $C_3$ and percentage unbound cortisol, showed no significant difference between the vaccinated and unvaccinated group. However, as a one-fifth dose of vaccine (which is effective in protecting ±80% of children against measles)[14-16] was administered, the effect of the dose recommended by the manufacturer on the cell-mediated immunity cannot be inferred from these results.

Although the aspects of PCM reported here returned to normal in the majority of patients within 4 weeks, it must be emphasised that complete recovery of the child is much more prolonged.

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