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Acute energy deprivation in man: effect on serum immunoglobulins antibody response, complement factors 3 and 4, acute phase reactants and interferon-producing capacity of blood lymphocytes

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SUMMARY

The effects of 10 days of total energy deprivation on serum levels of immunoglobulins, antibodies acute phase reactants and on interferon production were evaluated in fourteen healthy, normal-weight males. A significant depression was noted of the serum levels of complement factor 3, haptoglobin and orosomucoid. The titres of mercaptoethanol-sensitive specific antibodies to flagellin were higher in the subjects inoculated at the end of the starvation period than in controls and those inoculated at the start of the period. The serum levels of IgG, IgM, IgA, IgE, α-1-antitrypsin and complement factor 4, and the interferon-producing capacity of blood lymphocytes, were not changed. Thus, 10 days of total energy deprivation depresses the serum levels of several acute phase reactants and re-feeding may enhance antibody production.

INTRODUCTION

The prevalent association of undernutrition and infectious diseases has suggested a causal relationship. Studies of undernourished subjects have disclosed disturbances to various parameters of the defence against infectious agents (for a review, see Faulk, 1974).

Most of these studies have been carried out as field studies on subjects suffering from various forms of undernutrition. In these subjects noxious influences such as infections, climatic extremes, etc. may affect the variables under study (Kjellberg *et al.*, 1977; Murray & Murray, 1977). In an attempt to control such influences on, *inter alia*, immune functions, the effects of 10 days of complete energy deprivation (fasting) were studied under standardized conditions in healthy normal-weight males. The reactions of lymphocytes and polymorphonuclear granulocytes observed in this study have been reported earlier (Holm & Palmblad, 1976; Palmblad, 1976). We describe here the effects on humoral immunity measured as the serum levels of immunoglobulins and the specific antibody response to flagellin; on the complement factors 3 and 4 (C3 and C4), α-1-antitrypsin, haptoglobin and orosomucoid; and on the interferon-producing capacity of blood lymphocytes.

MATERIALS AND METHODS

The subjects and the experimental procedure have been described in detail elsewhere (Kjellberg *et al.*, 1977; Holm & Palmblad, 1976).

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Twenty healthy males participated in the study. After 4 'pre-starvation' days fourteen subjects were assigned to an experimental ('starving') group and six subjects to a control group. The control group continued on the standard food regimen throughout the study.

The experimental group was deprived of all food and allowed to drink only non-calorie beverages (not less than 3 litres) for the following 11 'starvation' days. After the 11 days of complete starvation, during which the subjects exhibited a mean weight loss of 6·4 ± 0·3 kg (range 8·5–5·4 kg), standard food was then gradually re-introduced during the following 4 days ('post-starvation period').

Venous blood samples were obtained on starvation days 1, 4 and 10, and 6 days after the discontinuation of starvation.

Serum was prepared from the blood samples and frozen at −20°C within 1 hr of taking it. The serum samples for determination of antiflagellin antibodies, immunoglobulins and complement levels were later transferred to −70°C. All serum samples from a single subject were analysed simultaneously for the determination of immunoglobulins, flagellin antibodies, complement factors and acute phase reactants.

5 μg of freshly depolymerized flagellin, batch K 7409 (Burroughs, Wellcome & Co., London, England), was given s.c. to one half of the starvation group (subgroup a), and the control group, on starvation day 1, and to the other half of the starvation group (subgroup b) on starvation day 10. Immediately prior to the administration of the flagellin, and 7 and 16 days later, blood samples were drawn for the determination of antiflagellin antibodies.

Serum immunoglobulins (IgG, IgM, IgA and IgE). These were determined by the Mancini method (Mancini, Carbonara & Heremans, 1965). The reference values were: for IgM, 0·26–1·22 g/l; for IgA, 0·67–3·27 g/l; for IgG, 7·06–15·30 g/l.

IgE was determined by the commercial RIST technique (Pharmacia Fine Chemicals, Uppsala, Sweden). The mean normal value was < 150 u/l.

Flagellin antibodies. The passive haemagglutination method described by Wistar (1968) was followed. Briefly, sheep red blood cells (SRBC) were treated with tannic acid and sensitized with flagellin (0·5 mg per ml). All sera were heat-inactivated and absorbed with SRBC. The agglutination titre was read in Perspex agglutination trays after 2 hr incubation at room temperature. Each serum, diluted 1:5, was tested with tanned and unsensitized SRBC. A serum with known titre was included as positive control. As a further control, sensitized cells were added to buffer only. 2-mercaptoethanol (ME) sensitive antibodies were determined after incubation with 0·1 M ME for 1 hr at 37°C prior to the haemagglutination assay.

Serum proteins. C3 and C4. These were determined by an electroimmunoassay (Laurell, 1972). The normal mean value, expressed as 100% (corresponding to 1·2 g/l for C3 and to 0·3 g/l for C4), was calculated from the results obtained by testing 200 blood donor sera. The reference values for C3 were 60–140% and for C4 40–200%.

Serum alpha-1-antitrypsin, haptoglobin and orosomucoid. These were determined by an automated immunoprecipitation test (Technicon, Clinical Methods No. 12, Geneva, Switzerland, 1974). The reference value for S-α-1-antitrypsin is 1·25–2·50 g/l, for S-haptoglobin 0·40–2·90 g/l, and for S-orosomucoid 0·40–1·10 g/l.

S-albumin. These levels were analysed with a routine bromcresol green method and haematocrite was analysed with a centrifuge method.

Interferon production. Interferon production was induced in the blood samples by adding 300 haemagglutination (HA) units of Sendai virus per ml as described previously (Strander *et al.*, 1970). After incubation for 24 hr the sera were separated, dialysed against buffered solution, pH 2, and assayed for interferon by the VSV plaque-reduction method (Strander & Cantell, 1967). Titres were expressed in terms of the unit assigned to the standard research preparation 69/19 (International Symposium on Standardization of Interferon and Interferon Inducers, London, 1969) and given per ml of serum. The number of interferon units obtained was related to the number of lymphocytes present in the blood samples.

Statistical analyses. These were performed utilizing the Student's two-tailed *t*-test. The paired *t*-test was used for the evaluation of significance of changes over time for both groups and the independent test for evaluating significance of differences between the groups, based on their deviation from the day 1 values.

RESULTS

Serum IgG, IgA, IgM and IgE

These remained unchanged during the study. No statistically significant differences were noted on any day between the starvation and the control groups.

Flagellin antibodies

The starving subjects who were inoculated on starvation day 10 (subgroup b) had a significantly higher mean antibody titre level than both the subjects forming subgroup (a) and the control group on post-inoculation day 7 ($P<0·001$ and 0·02, respectively) (Fig. 1). The antibody titres were reduced considerably after ME treatment. There were no differences between the mean titres of ME-resistant antibodies in the groups.

Serum proteins

In the starving subjects, the serum C3 level decreased on starvation day 10. The difference was statistically significant compared with the day 1 and 4 values (Fig. 2) ($P<0·001$ and 0·001, respectively) and

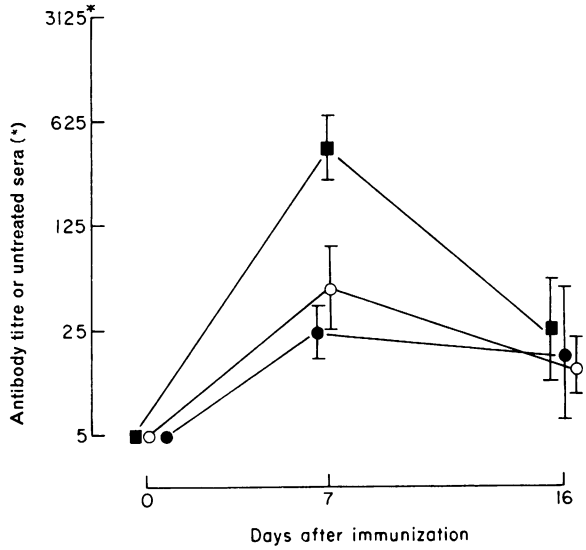

FIG. 1. Serum flagellin antibodies, mean values ± s.e.m. (●) starving subjects, subgroup (a); (○) controls (both groups inoculated on starvation day 1); (■) starving subjects, subgroup (b) (inoculated on starvation day 10).

with the day 10 value for the control subjects ($P<0{\cdot}001$). 6 days after the discontinuation of starvation, the mean level had increased, but was still statistically significantly lower than on starvation days 1 and 4 ($P<0{\cdot}05$ and 0·05, respectively) (Fig. 2). No subject showed values outside the normal range. No changes in the serum C4 level were recorded.

Serum haptoglobin and orosomucoid. These were significantly reduced on starvation day 10 ($P<0{\cdot}01$ and $P<0{\cdot}001$, respectively) (Fig. 3). No changes of serum α-1-antitrypsin were noted (Fig. 3).

Serum albumin. These levels showed small variations during the course of the study, which were parallel in the starving and control subjects.

Interferon production

The interferon production was not altered during the starvation period (Table 1).

Peripheral leucocyte counts

There were no statistical differences in lymphocyte, monocyte and granulocyte counts between the groups during the starvation period.

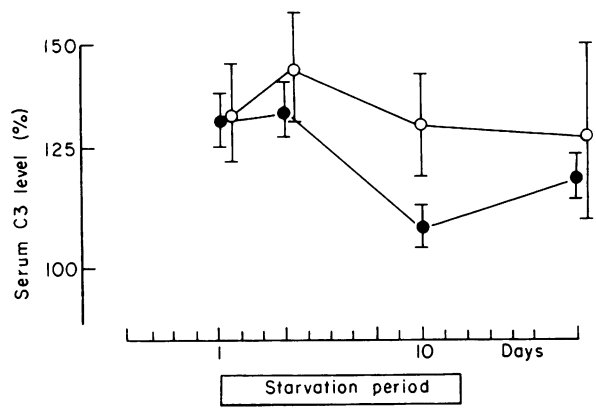

FIG. 2. Serum C3 levels, mean values ± s.e.m. (●) starving subjects; (○) controls.

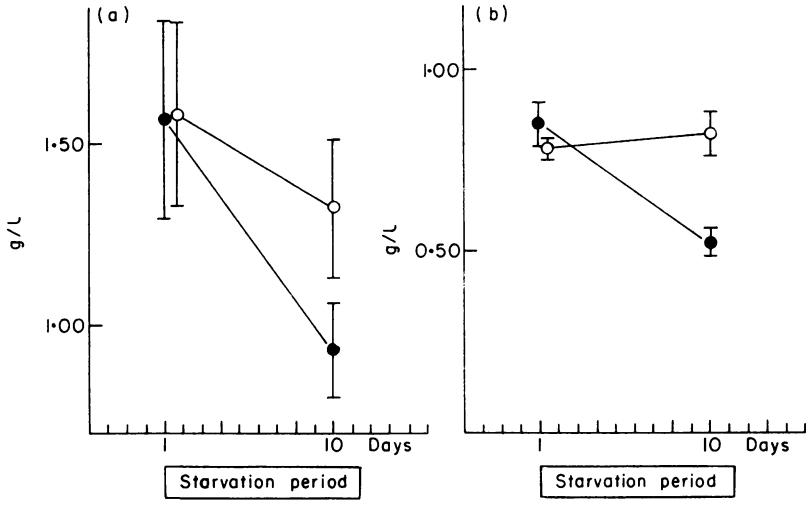

FIG. 3. Serum haptoglobin (a) and orosomucoid (b) levels. Mean values ± s.e.m. Symbols as in Fig. 2.

TABLE 1. Interferon-producing capacity (mean and s.e.m.)

	Starvation day						
	1		4		10		
Interferon units (IFU)	Starving subjects ($n = 7$)	Controls ($n = 6$)	Starving subjects ($n = 7$)	Controls ($n = 4$)	Starving subjects ($n = 7$)	Controls ($n = 6$)	P*
(IFU/ml)	921 ± 118	1250 ± 150	1179 ± 239	1700 ± 600	943 ± 219	1466 ± 250	n.s.
(IFU/10^6 lymphocytes)	325 ± 58	549 ± 65	547 ± 147	861 ± 388	324 ± 51	726 ± 169	< 0·05 (day 1) < 0·05 (day 10) (n.s.)

* Statistical significance of difference from controls on starvation days 1 and 10, respectively, and within brackets, from subjects day 1 value; n.s. = no significance.

Haematocrite

A small increase ($P < 0{\cdot}01$) was noted in the starvation group between starvation days 1 and 4, but no other changes occurred within or between the groups.

DISCUSSION

The exposure of healthy subjects to 10 days of energy deprivation was accompanied by a significant depression of the serum levels of C3, haptoglobin and orosomucoid. This occurred despite some increase of haematocrite, suggesting some haemoconcentration.

Haptoglobin, orosomucoid and C3 behave as acute phase reactants. Another substance belonging to this group, serum transferrin, also decreased in the starving subjects (Palmblad, 1976).

Decreased serum levels of acute phase reactants, such as siderophilin, transferrin and α-1-antitrypsin, have been reported previously in undernourished subjects (Chandra, 1972; Razban *et al.*, 1975). Increased levels of haptoglobin were also found, and they were attributed to ongoing infections. Thus,

acute energy withdrawal is accompanied by decreased levels of several acute phase reactants, of which C3 is one. However, the explanation for the decrease of these substances in serum during starvation is not known. Impaired synthesis, changes in the distribution between different compartments and increased catabolism may contribute. Previous reports from field studies in undernourished subjects have suggested that a decreased synthesis of serum complement is responsible for the low serum levels (Sirisinha *et al.*, 1973; Chandra, 1975; Razban *et al.*, 1975). Other studies on protein synthesis in undernourished subjects support such a hypothesis (Waterlow, 1968; Picou & Taylor-Roberts, 1969). However, in view of the rapid decline of the substances with a long $T\frac{1}{2}$, impairment of synthesis may not be the sole factor. Increased activation and/or catabolism may also contribute, especially in infected subjects (Chandra, 1975; Razban *et al.*, 1975). That a change in the distribution between intra- and extra-vasal compartments or serum dilution could be the cause was considered less likely (Sirisinha *et al.*, 1973).

The field studies on undernourished subjects have disclosed increased or normal levels of immunoglobulins (Watson & Freesemann, 1970; Chandra, 1972; Law, Dudrick & Abdou, 1973), and the increases have been attributed to the presence of infections (Chandra, 1972; Watson & Freesemann, 1970). An experimental situation comprising total energy deprivation without complicating infections may be accompanied by other changes of immunoglobulins. However, long-term experiments may be required to allow for changes within the IgG levels.

Immunization with flagellin resulted in significantly higher ME-sensitive antibody (IgM) titres in those immunized at the end of the starvation period and developing their antibodies during re-feeding. No depression of the antibody response by starvation was detected.

In field studies, undernourished subjects usually disclose a decreased capacity to mount an antibody response after immunization with keyhole limpet hemocyanine, flagellin, tetanus toxoid, typhoid, polio, measles and yellow fever vaccines, compared with various controls (Brown & Katz, 1966; Chandra, 1972, 1975; Law *et al.*, 1973; Mathews *et al.*, 1974; Coovadia *et al.*, 1974). The 10-day period of complete starvation in our study may be too short to depress the ability to produce antibody to flagellin. Moreover, under our experimental conditions, flagellin gave rise mainly to ME-sensitive antibodies, probably of the IgM class (Rowley & Mackay, 1969).

The present finding of an enhanced antibody synthesis during re-feeding may be of interest for the interpretation of results from field studies on the effect on antibody synthesis of food or protein supplementation to previously undernourished patients.

No results have been published previously concerning the capacity of circulating lymphocytes in undernourished subjects to produce interferon. The present findings indicate that 10 days of total energy deprivation do not alter this capacity. However, this exposure depressed their capacity to incorporate thymidine after stimulation with pokeweed mitogen and purified protein derivate (Holm & Palmblad, 1976). This finding, together with the changes reported here in the serum levels of certain proteins, may be of relevance to the impaired defence against infectious agents found in undernourished subjects.

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