Anorexia Nervosa and Polymorphonuclear (PMN) Granulocyte Reactions

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10 patients with anorexia nervosa were compared with controls with normal weight, regarding their peripheral blood polymorphonuclear (PMN) granulocyte reactions. The anorexia patients showed a statistically significant decrease in PMN bactericidal capacity and PMN adherence. The mean chemotaxis did not differ, but in two of the anorexia patients chemotaxis was almost absent. The intracellular activity of alkaline phosphatase was below the reference values in 5 of the 6 patients in whom it was investigated. - It is concluded that changes in granulocyte function may be noted in anorexia nervosa, but their clinical significance is uncertain, as no patients had recurrent or severe infectious diseases.

Key words: anorexia nervosa - cell adhesion - chemotaxis - granulocytes - leucocyte alkaline phosphatase - phagocytosis

Accepted for publication April 28, 1977

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Infections are common in chronically undernourished populations in the developing countries, and it has been suggested that the food deprivation is responsible for the increased susceptibility to infectious agents (Scrimshaw et al 1968, Vitale & Good 1974, Faulk 1974). The evaluation of a cause-effect relationship, however, is hampered by complex, concomitant environmental factors (Murray & Murray 1977, Kjellberg et al 1977).

Anorexia nervosa is a special form of undernutrition, existing in the industrialized countries. Although the intake of food, and consequently of energy, is greatly reduced in this disease, nothing definite is known about the incidence of infections. The sparse evidence available indicates that infections in general are uncommon and viral diseases even rare (Dally 1969). However, bacterial paronychia and staphylococcal skin infections may be frequent (Dally 1969). Some-
times, severe infections are the cause of death (Theander 1970, Warren & Wiele 1973).

A few recent studies have suggested that the defence against infectious agents might be affected in anorexia nervosa. Kjösen et al (1975) found a reduced leucocyte glucose oxidation, and there are case reports of a decreased bactericidal capacity of granulocytes (Gotch et al 1975) and low serum complement levels (Kim & Michael 1975).

As nothing could be concluded about the frequency of impaired granulocyte functions in anorexia nervosa, 10 consecutive patients have been studied with regard to functions of their peripheral blood polymorphonuclear (PMN) granulocytes.

**MATERIAL AND METHODS**

**Subjects**

9 girls and 1 boy with anorexia nervosa were studied. All of them conformed to the following criteria for the disease (chiefly from Dally 1969):

- age of onset before 35 years;
- active refusal to eat with accompanying weight loss;
- no evidence of schizophrenia, severe depression or organic disease.

The physical characteristics of the patients are given in Table 1. The mean weight loss was 27% from premorbid weight. All but one patient were below -2 SD on standard curves relating weight to height. They all showed classical symptoms of adaptation to starvation, such as bradycardia, hypotension and hypothermia. All 6 postpubertal girls had secondary amenorrhea and the male had no signs of puberty. None had a history of recurrent infections. All had blood PMN values above $1.0 \times 10^9\text{L}^{-1}$ (range 1.4–4.1). No anaemia or electrolyte disturbances were found and no patient was on a drug therapy.

20 controls with normal weight (14 females and 6 males) were investigated simultaneously. Details concerning them are also given in Table 1. They were members of the laboratory staff or blood bank donors, all apparently healthy and not on a drug regimen or pregnant.

**Bactericidal capacity**

The assay was performed essentially as described previously (Solberg 1972) but with a lower granulocyte concentration (Palmblad 1976, Palmblad et al 1977a). $2.5 \times 10^6$ (range 1.8–2.7) granulocytes per ml, obtained from heparinized venous blood, were mixed with pooled serum (10%) and sus-
pensions of Staph. aureus, grown over night (1.0 x 10^7 colony forming units, CFU, per ml). The tubes were incubated at 37° C and samples were removed after 22.5, 45 and 90 min of incubation for quantitation of viable CFU. The results are given as the percentages living CFU of the initial CFU counts. The reference values (mean ± 2 SD), obtained by analyzing the PMNs of 100 healthy blood bank donors (an extended control material), are, for the 90 min incubation period, < 5.6 % living CFU, mean 2.8 %. The results of the bactericidal test do not vary with differences in age and sex for groups relevant to this study (reviewed by Quie 1975).

Chemotaxis assay
The PMN chemotaxis was analyzed according to Hill et al (1975) and has been described in detail previously (Palmblad et al 1977a). Briefly, leucocytes were deposited on a 5 μm Millipore filter by centrifugation. The filters were placed in Boyden chambers, in which a bacterial chemotactically active factor, prepared from an E. coli strain, had been added to the attractant side. Controls (random mobility), were run simultaneously. After incubation for 3 h the numbers of PMNs that had migrated completely through the filter were determined by microscopy. The reference values (mean ± 1 SD) obtained by analyzing the PMNs of 44 healthy blood bank donors (an extended control material) are shown in Figure 2. The results of the chemotaxis assay do not vary with differences in age and sex (Hill et al 1975, cf Kretchmer et al 1976, Palmblad, unpublished results).

Adherence assay
The PMN adherence to nylon fibers was analyzed according to MacGregor et al (1974) and has described in detail previously (Palmblad et al 1977a). Briefly, when the number of band and segmented PMNs in heparinized blood had been counted samples were poured into 3 adherence columns (Pasteur glass pipettes in which 70 mg of scrubbed nylon fibers had been packed). The blood, having passed through the 3 pipettes, was pooled and the number of PMNs was counted. The results are given as the percentage of PMNs adhering to the nylon fibers. The reference value (mean ± 2 SD) obtained by analyzing the PMNs of 55 healthy blood bank donors (an extended control material) is 16.5-87 % (cf. Figure 3). No age or sex dependent variations have been observed in PMN adherence for groups relevant to this study (Ruley et al 1976, Palmblad, unpublished observations).

Activity of leucocyte alkaline phosphatase
Blood smears were stored at −20° C until staining, which was performed according to Cartwright (1968). The procedure has been described earlier (Palmblad 1976). Briefly, after determining the degree of alkaline phosphatase activity microscopically in each of 100 granulocytes, a total score was established by summing the individual cell scores, which ranged from 0' (no activity) to '4+' (intense activity). The reference values are: for men 22–124, and for women 33–149; there are no age-dependent differences for age groups relevant to this study (Kaplow 1968).

Statistical procedures were performed with Student's two-tailed t-test for independent data and linear regression analysis for the study of correlations between variables.

RESULTS

Bactericidal capacity
The anorexia patients had higher mean values than the controls for the percentage living CFU at 22.5, 45 and 90 min of incubation (p < 0.05 for all periods of incubation) (Table 2, Figure 1). The variation within the anorexia group was greater than for the controls. 4 of the 10 anorexia patients showed values above the reference value (mean ± 2 SD) for the 90 min incubation, established from the analysis of the extended control material. None of the controls showed values outside the reference value.

Chemotaxis
There was no statistically significant differ-
PMN FUNCTION IN ANOREXIA NERVOSA

### TABLE 2

**PMN bactericidal capacity, chemotaxis and adherence**
Mean ± SD values

<table>
<thead>
<tr>
<th></th>
<th>PMN bactericidal capacity</th>
<th>PMN chemotaxis</th>
<th>PMN random mobility</th>
<th>PMN adherence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% living CFU at</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.5 min</td>
<td>45 min</td>
<td>90 min</td>
<td></td>
</tr>
<tr>
<td>Anorexia patients</td>
<td>22.5 ± 7.21)</td>
<td>10.0 ± 5.51,2)</td>
<td>4.9 ± 4.11,4)</td>
<td>3.8 ± 2.63)</td>
</tr>
<tr>
<td>Controls</td>
<td>18.3 ± 3.9</td>
<td>7.1 ± 7.0</td>
<td>2.9 ± 1.2</td>
<td>6.4 ± 3.5</td>
</tr>
<tr>
<td>Extended control</td>
<td>19.1 ± 5.1</td>
<td>7.5 ± 3.1</td>
<td>2.8 ± 1.4</td>
<td>6.5 ± 3.3</td>
</tr>
<tr>
<td>material</td>
<td>n = 100</td>
<td>n = 100</td>
<td>n = 100</td>
<td>n = 44</td>
</tr>
</tbody>
</table>

1) Statistical significance of difference from controls p < 0.05
2) Statistical significance of difference from controls p < 0.001
3) Statistical significance of difference from extended control material p < 0.05
4) Statistical significance of difference from extended control material p < 0.001

ence (p > 0.05) between the mean values for the anorexia patients and the controls. However, chemotaxis was almost absent in 2 of the patients.

**Adherence**

The anorexia group showed significantly lower mean values than the controls (p < 0.001). 5 out of 10 anorexia patients exhibited adherence values below the reference value (mean -2 SD) and in 3 of them adherence was essentially absent.

**Alkaline phosphatase activity**

The activity could be estimated in 6 of the anorexia females. 1 had a score of 61, which is within the reference value, but the other 5 ranged between scores of 5 and 9, mean 6.

**Correlation analyses**

No significant correlation (p > 0.05) was found in the anorexia patients between, on the one hand, values for the bactericidal activity, chemotaxis and adherence, and on the other, for weight, and weight loss from premorbid weight. The variables reflecting PMN function did not correlate significantly (p > 0.05) with the others.

**DISCUSSION**

In the anorexia nervosa patients studied here, at least one of the following PMN functions was reduced: bactericidal capacity, adherence, chemotaxis or activity of alkaline phosphatase. The difference, compared with controls with normal-weight, was significant for PMN bactericidal capacity and adherence. Although the mean value for chemotaxis did not differ significantly between the two groups, there were 2 patients in whom the PMNs showed hardly any chemotaxis. The activity of alkaline phosphatase was markedly reduced in 5 of the 6 patients tested.

The pathogenic mechanisms that cause these differences in PMN functions between anorexia patients and controls have not been identified. However, some suggestions can be made against the background of other reports.

In another study (Palmblad et al 1977a) it has been demonstrated that decreases in
bactericidal capacity do not correlate significantly to PMN adherence. In view of this and other observations in the literature (Cline 1973, Washington 1976), it was con-

Figure 1. The PMN bactericidal test. Graphic representation of the percentages of living CFU for the anorexia patients and the control subjects.
sidered less likely that differences in PMN adherence can influence the bactericidal process in vitro. The present study supports this hypothesis, since no significant correlation was found between decreases in bactericidal capacity and adherence. Hence, taken together with the previous results, it is suggested that changes in PMN adherence, as measured here, cannot possibly be of importance for the results of the bactericidal process in vitro. Whether this is relevant for in vivo functions, too, remains to be determined.

In a previous study on PMN function in obesity (Palmblad et al 1977a) it was discussed whether an increased PMN adherence could influence the results of the chemotaxis assay used, resulting in increased chemotaxis values. However, no significant correlation was found between the values for the two variables, either in the previous study on obese patients, or in the present study. Regardless of these results, it is noteworthy that a mean increase in adherence was associated with a slight mean increase in chemotaxis and that decreased adherence accompanied a slightly decreased chemotaxis (cf Gallin & Wolff 1975, Keller et al 1975).

It has been emphasized that we measure only the function of the PMNs circulating in the blood. This circulating pool is in balance with a pool of PMNs marginating along the walls of blood vessels. It is not known whether the quantity and function of these pools differ in different conditions. Neither is it known whether such factors can contribute to some of the present differences between underweight and normal-
weight subjects. From the literature we know that the bone marrow may be hypocellular in anorexia nervosa (Mant & Faragher 1972). Such a finding suggests that the total granulocyte pool, including the circulating, could be reduced, too, sometimes resulting in neutropenia (Mant & Faragher 1972). Also it should be noted that granulocytes perform most of their 'work' in the tissues and not in the circulating blood, and that the quantity and function of these granulocytes are unknown. In fact, it has been suggested that a redistribution of cells could contribute to the neutropenia sometimes observed in anorexia nervosa (Mant & Faragher 1972).

On the other hand it can be argued that a decreased adherence could diminish the tendency of circulating granulocytes to adhere to blood vessel endothelium and, accordingly, to enter the tissues. The relevance of the in vitro adherence process to the in vivo conditions has been reviewed elsewhere (Brubaker 1974). Further, the accumulation of granulocytes in an infectious focus could be reduced if chemotaxis is impaired. That such in vitro findings as those presented here are relevant to in vivo chemotaxis has been demonstrated (Jones et al 1977).

As in the study on PMN function in obesity (Palmblad et al 1977a), we did not find a significant correlation between body wt. and PMN function in anorexia nervosa. Neither was weight loss significantly correlated to PMN function. However, the opposite has been found in a case report; that is to say, an increase in weight accompanied the normalization of PMN bactericidal capacity in an anorexia patient (Gotch et al 1975).

In the present study there was no significant correlation between the variables reflecting various PMN functions. As all patients had a normal value for at least one function, it may be hypothesized that the part of the antimicrobial defence system, attended to by the granulocytes, is only partly impaired. This could constitute an important difference between anorexia patients, in whom infections are not remarkably common despite a markedly reduced food intake, and chronically undernourished subjects in the developing countries, in whom infections are common. In the latter, decreased cell-mediated and humoral immunity has also been demonstrated, in addition to decreased granulocyte function (Faulk 1974, Vitale & Good 1974). It remains to be seen whether changes in cell-mediated and humoral immunity also occur in anorexia nervosa.

Another explanation for the rarity of infections in anorexia nervosa patients may lie in differences in the quality of the energy intake compared with conditions in developing countries. The latter may involve a lack of all nutrients and the protein intake is most affected. In contrast, undernutrition in anorexia nervosa is often characterized by a marked deficiency of carbohydrate and energy, but not of protein and fat (Russell 1967, Dally 1969, Mant & Faragher 1972). Also iron and folic acid deficiencies are not marked in anorexia nervosa, but are so in subjects from developing countries (Mant & Faragher 1972, Chandra 1976).

In this context it may be relevant to consider some results from animal studies concerning the effect of energy deprivation on host defence. Cooper et al (1974) and Good et al (1976) have reported that reduction of protein intake enhanced cell-mediated immune responses, resistance to viral infections and to the development of certain malignant diseases but decreased resistance to
bacterial infections. Also, acute total food deprivation for 10 d was accompanied by decreases in PMN bactericidal capacity and activity of alkaline phosphatase (Palmblad 1976), lymphocyte DNA-synthesis (Holm & Palmblad 1976) and serum levels of several acute phase proteins (Palmblad et al 1977b) without any sign of increased susceptibility to infectious agents (Kjellberg et al 1977). In fact, it has been argued that food deprivation per se does not inevitably lead to an increased susceptibility and that refeeding of starving subjects might activate infections (Murray & Murray 1977). However, it should be strongly emphasized that fasting in normal-weight persons has not been convincingly associated with increased health.

Finally, the clinical significance of the alterations to PMN function demonstrated here is uncertain, since none of the patients had recurrent, unduly severe or protracted infectious diseases. In other conditions, impairment of bactericidal functions has been shown to accompany e.g. some inherited diseases, where infectious complications are frequent (Quie 1975).

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (No 19P-4316) and the Swedish National Defence Research Institute. The skilful technical assistance of Mrs. Clary Hedlund is gratefully acknowledged.

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