Phagocytic and alkaline phosphatase activity of leukocytes in kwashiorkor

Eight female children, 3 to 5 years of age, with kwashiorkor, were studied longitudinally from time of admission until complete recovery. The alkaline phosphatase activity of the leukocytes and the opsonocytophagic index were investigated. Alkaline phosphatase activity was relatively high on admission because of, presumably, concurrent infection. There was a slight tendency to decrease during recovery. In two cases in which intercurrent infections developed during hospitalization, a definite increase in the alkaline phosphatase activity of the leukocytes was observed. It is suggested that this reaction should be investigated for its possible value in revealing infectious activity in the absence of leukocytosis. No change in the opsonocytophagic index was detected on admission or following recovery.

C. Tejada, M.D.,* V. Argueta, M.D., Margarita Sánchez, M.T. (A.S.C.P.), and C. Albertazzi

GUATEMALA CITY, GUATEMALA, CENTRAL AMERICA

SEVERAL nutritional deficiencies apparently depress the reticuloendothelial system, producing a diminution in the number of cells released into the bloodstream, a reduction in antibody production, and a decrease in phagocytic activity as well as other functions; the possible role played by phagocytic activity in the interrelationship between nutrition and infection has been reviewed by Scrimshaw, Taylor, and Gordon.[1] Few studies, however, have measured the effect of nutritional status on metabolic parameters which characterize the leukocytes in either man or experimental animals.

Children with kwashiorkor are very susceptible to infections and often die as a con-

From the Institute of Nutrition of Central America and Panama (INCAP) Guatemala, Central America, the Department of Pediatrics, General Hospital, Guatemala, Central America, and the School of Medicine, University of San Carlos de Guatemala.

This investigation was partially supported by Grants AM-981 and AM-4827 from the National Institutes of Health.

INCAP Publication I-271.

**Address, Division of Pathology, Institute of Nutrition of Central America and Panama (INCAP), Apartado Postal No. 11-88, Guatemala, Central America.*

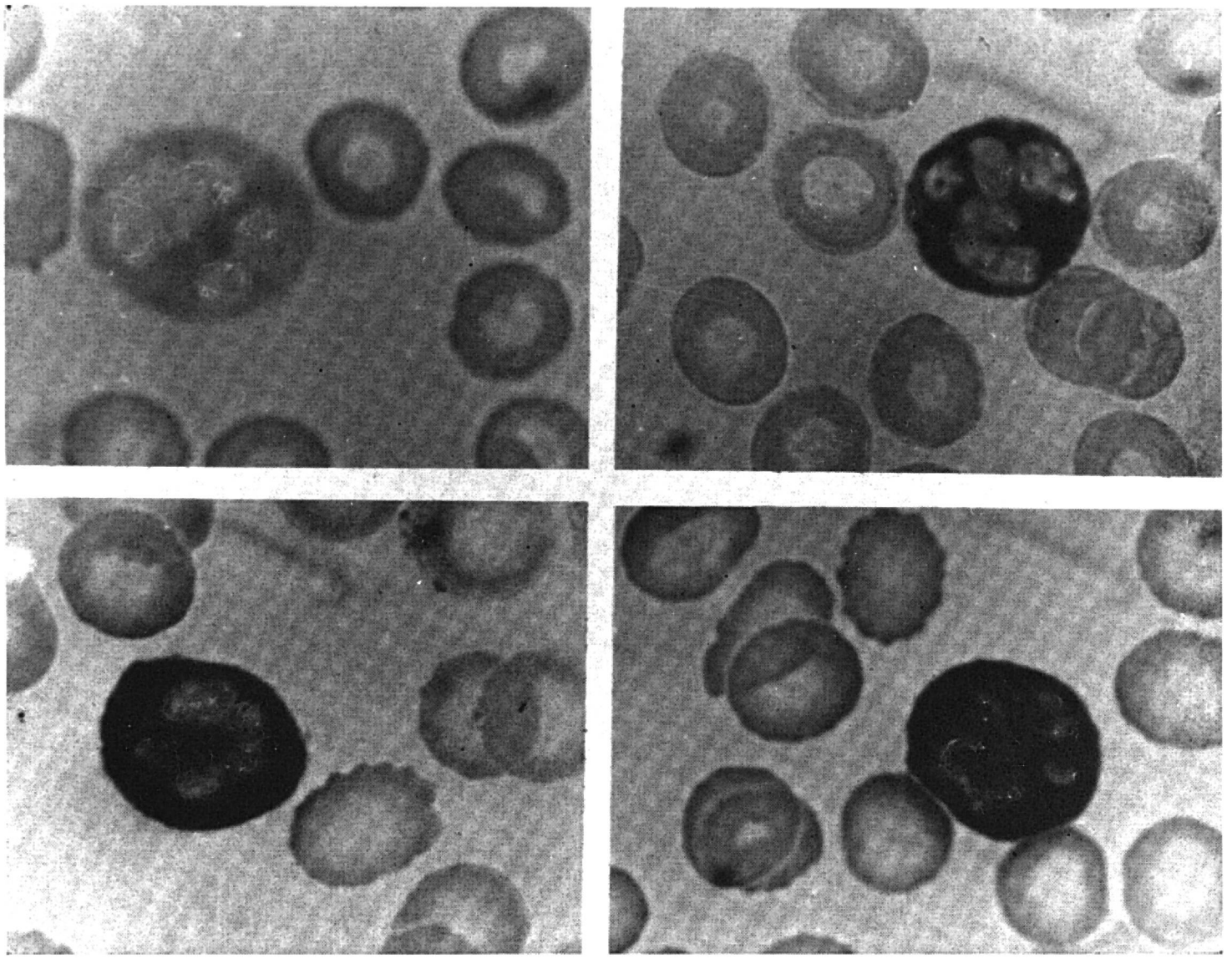

Fig. 1. Leukocytes with different degrees of activity. Upper left, Grade 1; upper right, Grade 2; lower left, Grade 3; lower right, Grade 4.

sequence of bronchopneumonia which is difficult to recognize clinically until it is manifested terminally by respiratory insufficiency.[2] There is no clear explanation, however, as to how kwashiorkor predisposes to infections.

Infection, on the other hand, may influence any stage in the natural history of kwashiorkor. It may serve to precipitate and aggravate the acute episode or interfere with recovery. It is not unusual during infectious episodes to observe a decrease in food intake and increased nitrogen loss in the urine.[3]

Among the mechanisms of body defense against infection that could be adversely affected by kwashiorkor, probably antibody formation and phagocytic activity are the most important. Other possible factors cited by Scrimshaw[1] are: the direct effect of malnutrition on tissue integrity, interference with the synthesis of nonspecific protective substances, nonspecific destruction of bacterial toxins, alterations of intestinal flora, and changes in the endocrine balance of the host.

Antibody formation in protein malnutrition has been studied by Wohl, Reinhold, and Rose[4] and Olarte, Cravioto, and Campos[5] who showed that protein deficiency may adversely affect antibody synthesis. Very few studies, however, have been carried out showing the effect of the same deficiency on leukocytic activity and production. Guggenheim and Buechler[6] in their work with rats concluded that low-protein diets interfere with leukocytic production.

Children with kwashiorkor provide a unique opportunity to study the effect of severe protein deficiency on leukocytic activity and production. The present study was designed to investigate the alkaline phosphatase and phagocytic activity of the leukocytes during the acute stage of kwashiorkor and to follow the same children during recovery.

MATERIAL AND METHODS

Eight female children, 3 to 5 years of age, admitted to the Pediatric Department at the General Hospital in Guatemala City, with

advanced kwashiorkor, were studied. The most prominent clinical signs and symptoms were pitting edema mainly of the lower extremities, a variable degree of skin changes from mild hyperkeratosis to severe pellagroid dermatosis, poor implantation, dryness, and decoloration of the hair, apathy, irritability, anorexia, and growth arrest.

Diarrhea prior to and/or during admission was a constant finding in the eight children and varied from being mild in 6 cases to severe in the other 2. The latter apparently were of frank infectious origin, accompanied by abdominal colic, tenesmus, and bloody mucous stools. The diet before the beginning of the illness was deficient in protein and relatively rich in carbohydrates. Animal protein intake was in general very low. The principal foods in this diet were black beans, rice, tortillas (corn), and bread; only one of the patients was accustomed to milk and said to drink a glass of this daily; 2 of the children ate a piece of cheese or an egg sporadically.

In spite of the edema, the weight and size of all the children were much below the normal averages. Every child was treated with a diet consisting basically of skim milk plus the ordinary pediatric solid food diet given to other patients and supplemented with multiple vitamins. This diet enhanced prompt recovery. Parenteral fluid therapy was used to correct the hydroelectrolytic imbalance usually observed on admission.

One child who had anemia also received 400 c.c. of blood. Another child with severe diarrhea was treated with 750 mg. chloroamphenicol daily for 9 days and 800,000 units of penicillin daily for 10 days. The other children received no antibiotic therapy.

The children studied were followed from admission to recovery. During the first 2 weeks, analysis of the phagocytic activity and the alakaline phosphatase activity of the leukocytes, as well as total serum protein, total and differential white blood cell counts, was carried out every other day. After this, they were performed weekly. When a child developed fever or gave evidence of some infectious process, tests were resumed every other day until the signs and symptoms disappeared.

Disappearance of clinical symptoms, an increase in total serum proteins, and weight gain were the criteria used as indices of recovery. The study was discontinued when the children were clinically well and the serum proteins had been stabilized within normal limits for at least 3 weeks.

The method used for studying phagocytic activity was essentially the same as that described by Balch and Spencer,[7] but modified by us as a result of the observations of Berry and Derbyshire,[8] Berry, Starr, and Haller[9] and Stitt, Clough, and Branham.[10]

An 18 hour culture of nonpathogenic *Staphylococcus albus* was used. To eliminate

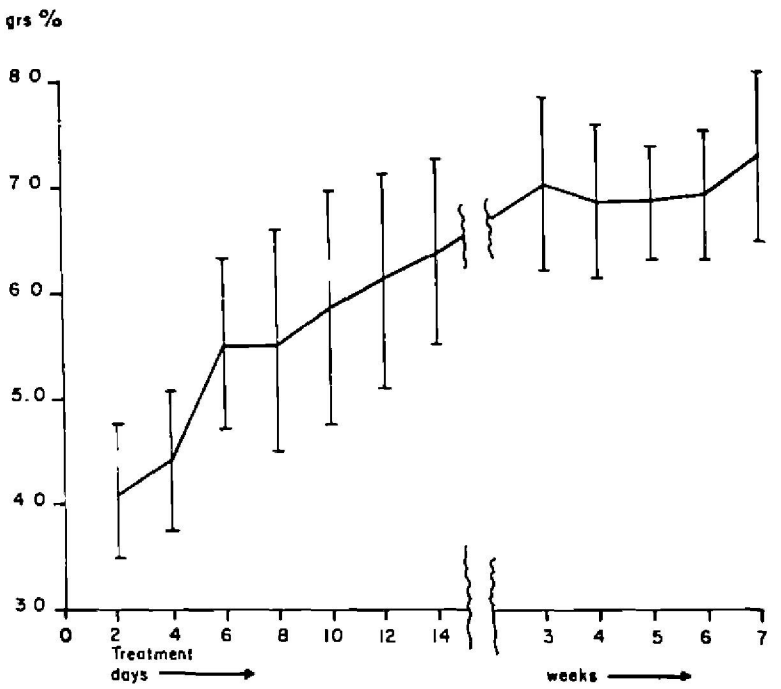

Fig. 2. Average serum total proteins in 8 children during recovery from kwashiorkor.

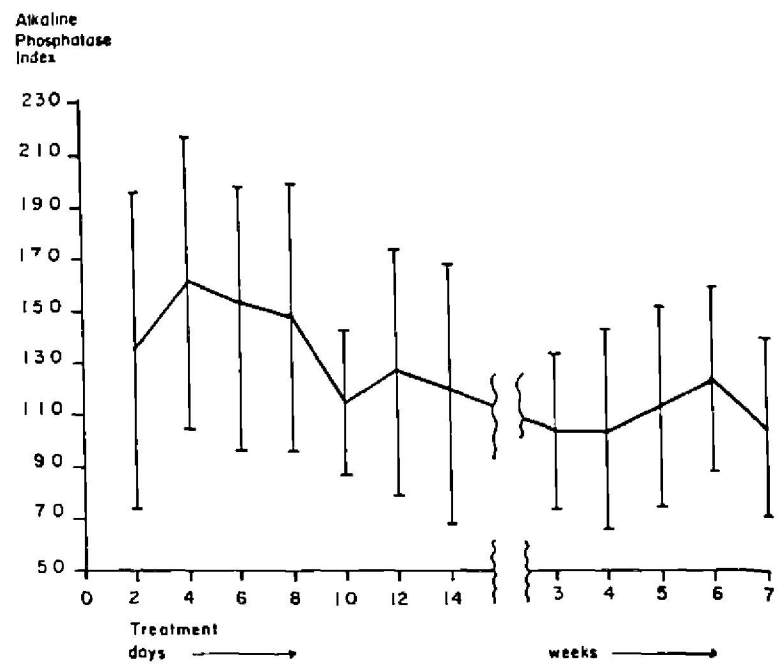

Fig. 3. Average alkaline phosphatase index of neutrophilic leukocytes in 8 children recovering from kwashiorkor.

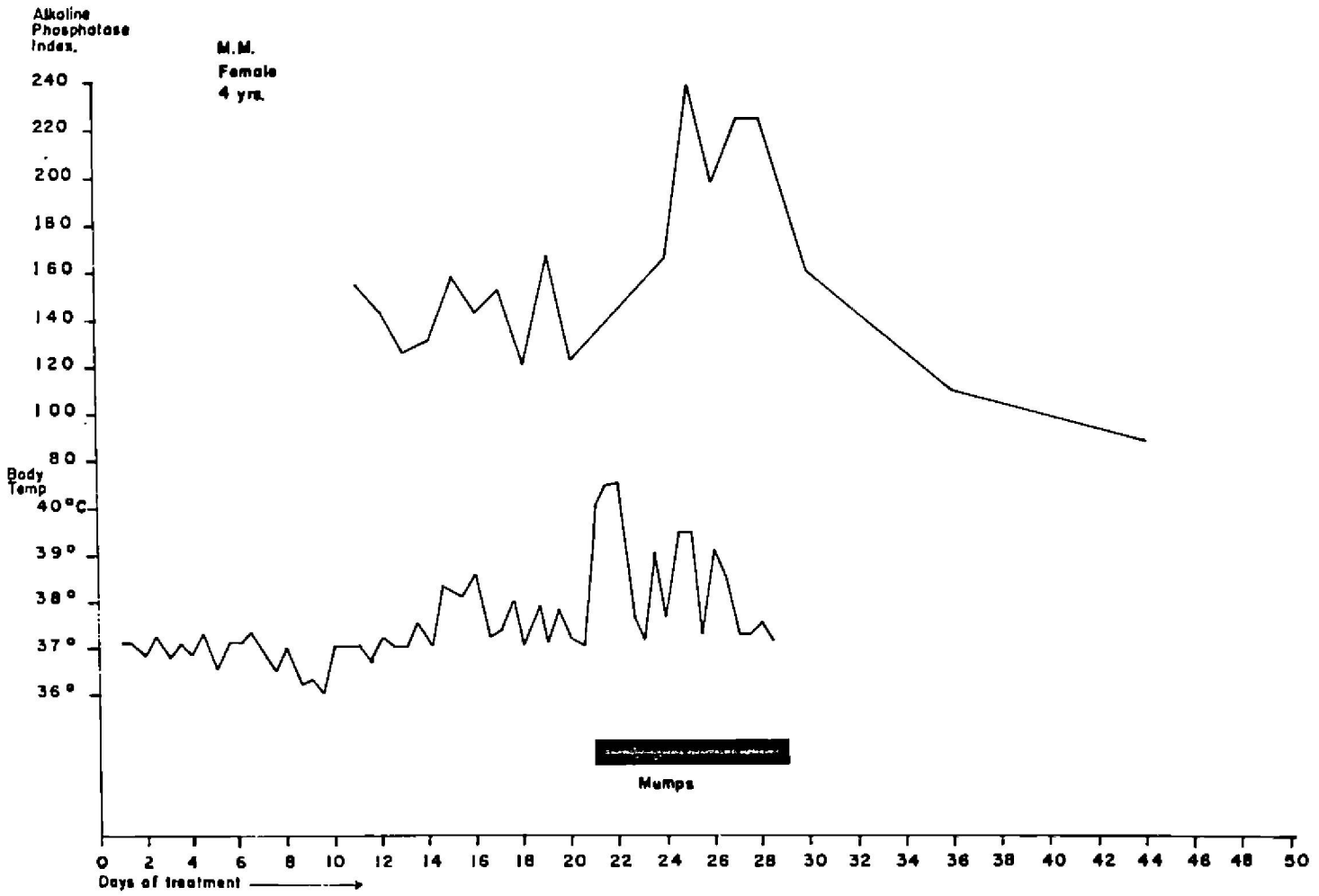

Fig. 4. Alkaline phosphatase index and infectious episodes in a child recovering from kwashiorkor.

pathogenicity as a variable, *S. albus* was selected instead of *S. aureus*. The culture was suspended in fresh 0.85 per cent saline solution and agitated in an electric shaker in order to separate all the bacterial clumps and to insure uniformity of suspension. It was then diluted to give a light transmittance of 70 per cent in a Coleman Jr. spectrophotometer with the use of a 650 mμ wave length.

Blood was heparinized (0.11 mg. of heparin per milliliter of blood) and kept cool until tested. The time between the collection of the blood and the performance of the test was never greater than 6 hours. One-tenth milliliter of blood was mixed thoroughly with 0.01 of bacterial suspension and incubated in a 37° C. water bath for exactly 30 minutes. The suspension was mixed thoroughly again, smears were prepared on coverslips and after air drying, were stained by the May-Grünwald-Giemsa method and mounted.

The phagocytized staphylococci were counted in one hundred consecutive neutrophils. The average number of staphylococci per cell was reported as the opsonocytophagic index.

Alkaline phosphatase activity of the white blood cells was studied according to the method described by Kaplow[11] modified by Koler and associates.[12] Smears were prepared by the coverslip technique.

Within 2 hours after bleeding, the coverslips were fixed for 30 seconds in a formalin-methyl alcohol mixture (10 c.c. formaldehyde, 90 c.c. methyl alcohol), washed immediately for 10 seconds in running tap water, air dried, and kept in the refrigerator. The activity was determined within a week after the specimens were taken. The smears were incubated in a substrate mixture for exactly 10 minutes in a 37° C. water bath. This substrate mixture is prepared with 0.05 M propanediol buffer, pH 9.75, which contains sodium alpha naphtyl acid phosphate and fast blue RR. The smears were then washed immediately in running tap water, counterstained in Mayer's hematoxylin for 3 or 4 minutes, and mounted in glycerol gelatin.

One hundred consecutive neutrophils were then read in each smear, and rated from 0 to 4 according to the intensity of the blackish brown deposit in the cytoplasm. The sum of

all the ratings is the score or alkaline phosphatase index.

Fig. 1 shows various examples of leukocytes with different degrees of activity. Those with no activity have a cytoplasm which is paler in color than the surrounding red blood cells. Leukocytes rating one degree of activity have in their cytoplasm the color of the surrounding red blood cells or one slightly darker. A rating of two shows a fine granular brownish black deposit in the cytoplasm. Those with heavy granular precipitate have a rating of 3; cells with a rating of 4 have such a dense deposit in the cytoplasm that the nucleus is difficult to see.

Total serum proteins were determined by the method described by Lowry and Hunter.[13]

RESULTS

Fig. 2 shows the average serum proteins with the standard deviation. Total proteins varied rapidly from the day of admission, reaching a plateau at the third week. On admission the average protein was 4.1 Gm. per 100 ml. and on discharge 7.3 Gm. per 100 ml. The difference is highly significant ($P < 0.01$). Serum total protein has been used in many previous investigations as an index of recovery from protein malnutrition and it parallels the weight gain and disappearance of the clinical symptoms.

Fig. 3 presents the average and standard deviation of the alkaline phosphatase index. Because of the high variability observed in this determination and the relatively small number of cases, it was not possible to establish any real differences that could be attributed to the effect of recovery on the alkaline phosphatase activity. In a preliminary way, however, this curve suggests a slight tendency to a decrease in the alkaline phosphatase activity throughout the child's recovery period. The standard deviation also tends to decrease with recovery.

The average alkaline phosphatase activity on admission was higher (136 units S.D. ± 60.9) in the experimental group than in 13 well-nourished control children of the same age and sex from high income families. In this control group the alkaline phosphatase index was 71 (S.D. ± 21.5). The statistical difference, however, was not significant, again due probably to the relatively small number of cases and the high variability of the results.

On discharge, the average alkaline phosphatase index was 98 (S.D. ± 38); a value

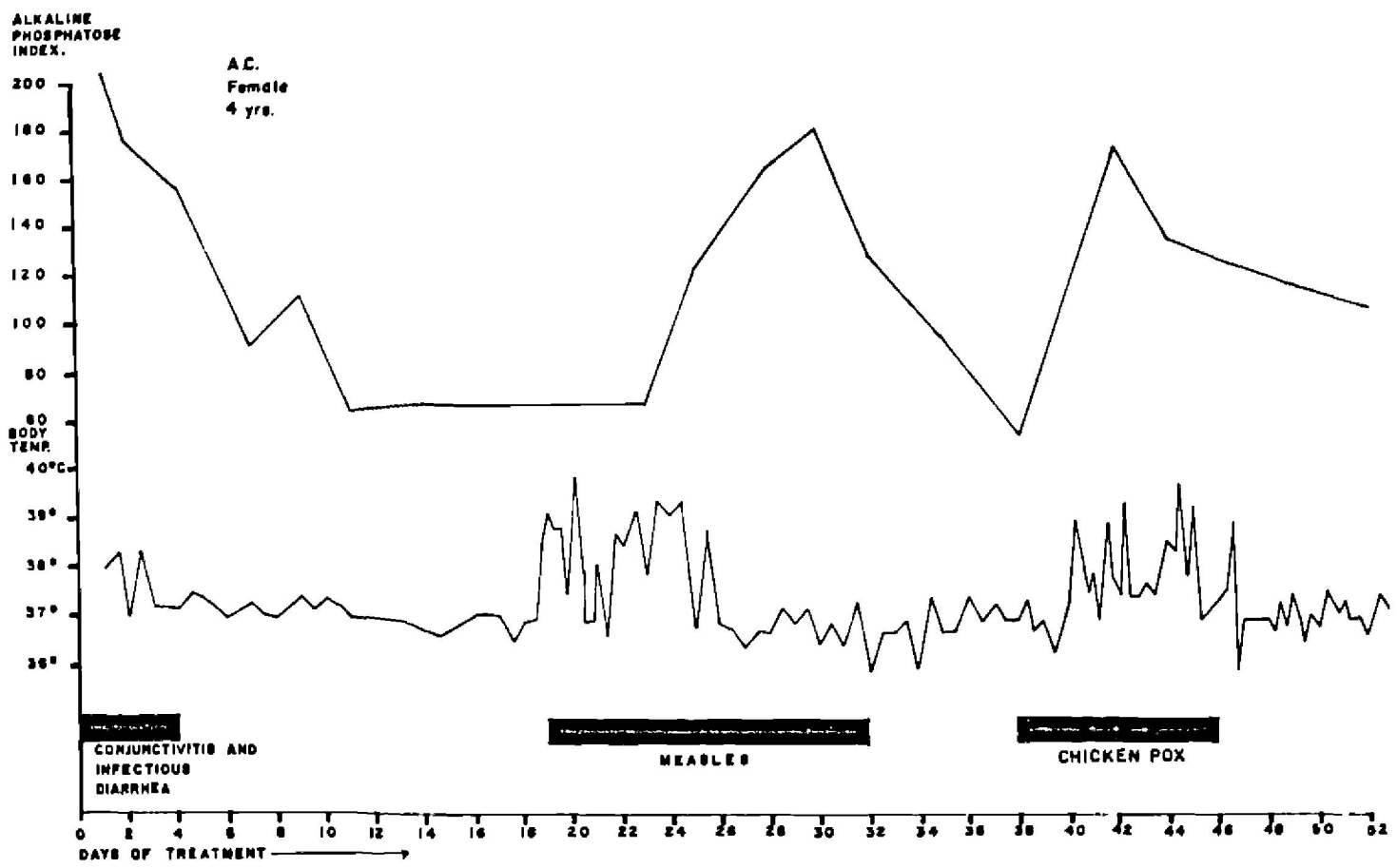

Fig. 5. Alkaline phosphatase index and infectious episodes in a child recovering from kwashiorkor.

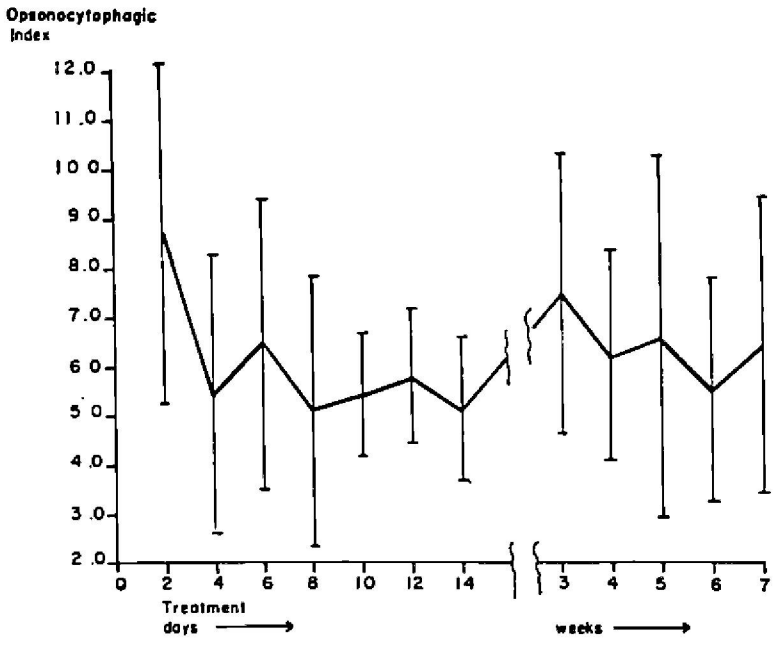

Fig. 6. Average opsonocytophagic index in 8 children recovering from kwashiorkor.

closer to the one observed in well-nourished controls.

It is well known that leukocytes in the bloodstream show a constant and strong alkaline phosphatase reaction in patients suffering from different infectious diseases. The development of an infectious episode in 2 of the children studied during hospitalization presented a good opportunity to study the effect of the infection on the alkaline phosphatase index of the leukocytes before, during, and after the infection, as well as the type of reaction observed in children with a poor nutritional background.

Figs. 4 and 5 present the alkaline phosphatase activity of the leukocytes in these two children. One child (M. M.) developed a temperature up to 38.4° C. on the seventeenth day of hospitalization and on the twenty-third day the temperature went up to 40.5° C. The child presented salivary gland swelling, and a diagnosis of epidemic parotitis was made. As seen in Fig. 4, the alkaline phosphatase index increased to 236 on the twenty-fourth day. Before the infection, the values ranged between 120 and 146.

The other child (A. C.) had 3 febrile episodes during her hospitalization. She came to the hospital with infectious diarrhea and acute conjunctivitis. Later, after being transferred to a convalescent home, she developed measles which lasted from the twenty-third to the thirty-second day; 6 days after recovery from this, she developed chickenpox. The alkaline phosphatase activity of the leukocytes, as seen in Fig. 5, increased during the three infectious episodes described.

Fig. 6 shows the average opsonocytophagic index of the neutrophils at different intervals during the entire period of hospitalization. The variability is such that if there is some effect due to treatment and, therefore, recovery, it is undetectable. Statistical analysis showed no significant difference between the different intervals. There again was no difference between the same children upon admission (poorly nourished) and upon discharge (well-nourished).

Contrary to alkaline phosphatase activity, phagocytic activity was not observed to alter in the presence of an infectious episode.

DISCUSSION

Malnutrition in either human beings or animals is known to decrease resistance to many bacterial infections.[1] Among the possible mechanisms accounting for this synergism, probably the most important are lowered antibody response, altered tissue integrity, and decreased phagocytic activity of the reticuloendothelial system. Juhlin,[14] working with starved rabbits, showed a decreased capacity of the Kupffer cells of the liver to phagocytize particles. A number of investigators have also found that severe nutritional deficiencies, including protein depletion, may result in decreased phagocytic activity of the leukocytes.[15-20] The restoration of phagocytic activity as nutritional status improved was interpreted by Cottingham and Mills[18] as being caused by the release of new phagocytes produced from the bone marrow.

The complete lack of apparent change in the opsonocytophagic index during recovery from kwashiorkor, as observed in the present work, appears to be contrary to the results of other studies cited. Some workers have also failed to find any change in the opsonocytophagic index in undernourished patients. Bieler, Ecker, and Spies[21] examining 8 patients with hypoproteinemia and anemia of a nutritional origin, observed normal complement titers, antisheep hemagglutinin titers, and opsonic indices. Balch and Spencer[22] also

found no significant difference in the opsonocytophagic index after comparing 17 well-nourished adult subjects with 20 who had advanced wasting disease. In both these studies, the total serum proteins of the patients were much higher (5.1 Gm. and 6.4 Gm. per 100 ml., respectively) than in the children studied in the present experiment.

From this study, it is possible to suspect that in the acute stage of kwashiorkor, circulating leukocytes are well matured as indicated by the A. P. activity and are able to phagocytize bacteria. This effect is also consistent throughout the recovery period. Therefore, the defense mechanism that is altered in malnutrition is probably related not to the circulating leukocytes, but to the mechanism of production and reactivity of the same cells at the tissue level.

Berry and colleagues[23] have reported increased phagocytic activity in anemic patients in comparison with subjects possessing normal hematologic values. In the present study the children showed a mild degree of anemia. No correlation, however, was observed between the phagocytic activity of leukocytes and the anemia observed. Balch and Spencer[22] also failed to confirm the observations of Berry and associates.[23]

This study shows that phagocytosis, at least in vitro and of nonpathogenic staphylococci, is normal, regardless of the degree of malnutrition. It is quite possible that in kwashiorkor the leukocytes already circulating are well matured. Cottingham and Mills[18] postulated that mature phagocytes in the circulating blood seem to be less affected by a change in the nutritional status of the individual. If this holds true, the possibility remains that patients with kwashiorkor may not have the capacity to produce sufficient numbers of neutrophils in response to infection, although the circulating leukocytes have normal phagocytic activity. It is well known that many children with kwashiorkor have leukopenia even in the presence of severe infection.[2]

The alkaline phosphatase activity of the leukocytes could be an indication of chemical maturity of these cells. Histochemical and biochemical methods have demonstrated marked differences in enzyme activity between cells that are morphologically indistinguishable.

Although the exact function of alkaline phosphatase in the neutrophil is still unknown, it appears to be involved in glycogenesis and nucleic acid formation.[24] This leukocyte enzyme is able to hydrolyze phosphates not only with sodium β glycerophosphate as substrate, but also by the metabolically important phosphate ester, adenosine-5′-phosphate and glucose-1-phosphate.[25]

Decreased alkaline phosphatase activity of the granulocytic leukocytes has been observed in both acute and chronic myelocytic leukemia,[11, 12, 26] and has been proposed as a laboratory method to differentiate these conditions from other leukemoid reactions.[12] Increased alkaline phosphatase activity has also been observed in stressful conditions[27] such as operative procedures, traumas, hemorrhage, pregnancy, and myocardial infarction. Valentine and co-workers[27] were able to increase the enzymatic activity of leukocytes by injecting highly purified ACTH gel and to a lesser extent by using cortisone acetate and hydrocortisone; this suggests a possible relation between leukocytic alkaline phosphatase activity and pituitary and/or adrenal cortical excretion.

In the present work, alkaline phosphatase activity was high at the time of admission, but there was a tendency to decline with treatment; the difference, however, was not statistically significant. It is possible that this slight difference is related to the frequent infections, mainly diarrhea, observed on admission.

Many children with kwashiorkor during the period of recovery develop fatal infections, chiefly bronchopneumonia, which are clinically asymptomatic until respiratory insufficiency occurs.[2, 28] Many appear without any leukocytosis and in some cases even with leukopenia.[2] Two of the children studied showed increased alkaline phosphatase activity during their infectious period while hospitalized. This indicates that alkaline phosphatase activity of the leukocytes may

possibly be used as a test to detect any asymptomatic infections which may occur in children recovering from kwashiorkor, especially in cases which run without fever or leukocytosis. However, further investigation is necessary in order to evaluate and establish the consistency and practicability of this method.

SUMMARY

Eight female children, 3 to 5 years of age, with kwashiorkor, were studied longitudinally from time of admission until complete recovery. The alkaline phosphatase activity of the leukocytes as well as the opsonocytophagic index was investigated. Alkaline phosphatase activity was relatively high on admission due, presumably, to concurrent infection. There was a slight tendency to decrease during recovery. In two cases, in which intercurrent infections developed during hospitalization, a definite increase in the alkaline phosphatase activity of the leukocytes was observed. It is suggested that this reaction should be investigated for its possible value in revealing infectious activity in the absence of leukocytosis.

No change in the opsonocytophagic index was detected on admission or following recovery.

REFERENCES

1. Scrimshaw, N. S., Taylor, C. E., and Gordon, J. E.: Interactions of nutrition and infection, Am. J. Med. Sc. **237:** 367, 1959.
2. Tejada, V. C., Béhar, M., and Cofiño, E.: Estudio clínico patológico de las bronconeumonías del niño desnutrido, Rev. Col. Med. Guatemala **7:** 134, 1956.
3. Wilson, D., Bressani, R., and Scrimshaw, N. S.: Infection and nutritional status. I. The effect of chicken pox on nitrogen metabolism in children, Am. J. Clin. Nutrition **9:** 154, 1961.
4. Wohl, M. G., Reinhold, J. G., and Rose, S. B.: Antibody response in patients with hypoproteinemia—with special reference to the effect of supplementation with protein or protein hydrolysate, Arch. Int. Med. **83:** 402, 1949.
5. Olarte, J., Cravioto, J., and Campos, B.: Inmunidad en el niño desnutrido. I. Producción de antitoxina diftérica (comunicación preliminar), Bol. med. Hosp. inf. (México) **13:** 467, 1956.
6. Guggenheim, K., and Buechler, E.: The effect of quantitative and qualitative protein deficiency on blood regeneration. I. White blood cells, Blood **4:** 958, 1949.
7. Balch, H. H., and Spencer, M. T.: Phagocytosis by human leucocytes. I. Effect of fibrin on phagocytosis of staphilococci and of encapsulated pneumococci by normal human leucocytes, J. Clin. Invest. **33:** 1314, 1954.
8. Berry, L. J., and Derbyshire, J. E.: Effect of malonate on selected body defenses, Proc. Soc. Exper. Biol. & Med. **92:** 315, 1956.
9. Berry, L. J., Starr, R. W. and Haller, E. C.: The effect of surface-active agents on phagocytosis, J. Bact. **57:** 603, 1949.
10. Stitt, E. R., Clough, P. W., and Branham, S. E.: Practical bacteriology, hematology and parasitology, ed. 10, Philadelphia, 1948, The Blakiston Company, p. 290.
11. Kaplow, L. S.: A histochemical procedure for localizing and evaluating leucocyte alkaline phosphatase activity in smears of blood and marrow, Blood **10:** 1023, 1955.
12. Koler, R. D., Seaman, A. J., Osgood, E. E., and Vanbellinghen, P.: Myeloproliferative diseases. Diagnostic value of the leukocyte alkaline phosphatase test, Am. J. Clin. Path. **30:** 295, 1958.
13. Lowry, O. H., and Hunter, T. H.: The determination of serum protein concentration with a gradient tube, J. Biol. Chem. **159:** 465, 1945.
14. Juhlin, L.: The effect of starvation on the phagocytic activity of the reticulo-endothelial system, Acta physiol. scandinav. **43:** 262, 1958.
15. Berry, L. J., Davies, J., and Spies, T. D.: The relationship between diet and the mechanisms for defense against bacterial infections in rats, J. Lab. & Clin. Med. **30:** 684, 1945.
16. Cannon, P. R.: The importance of proteins in resistance to infection, J. A. M. A. **128:** 360, 1945.
17. Cottingham, E., and Mills, C. A.: Influence of environmental temperature and vitamin-deficiency upon phagocytic functions, J. Immunol. **47:** 493, 1943.
18. Cottingham, E., and Mills, C. A.: Timing of phagocytic changes in malnutrition, J. Lab. & Clin. Med. **30:** 498, 1945.
19. Effect of nutrition on phagocytosis, Nutrition Rev. **2:** 232, 1944.
20. Gellhorn, E., and Dunn, J. O.: Undernutrition, starvation and phagocytosis, J. Nutrition **14:** 145, 1937.
21. Bieler, M. M., Ecker, E. E., and Spies, T. D.: Serum proteins in hypoproteinemia due to nutritional deficiency, J. Lab. & Clin. Med. **32:** 130, 1947.
22. Balch, H. H., and Spencer, M. T.: Phagocytosis by human leucocytes. II. Relation of nutritional deficiency in man to phagocytosis, J. Clin Invest. **33:** 1321, 1954.

23. Berry, L. J., Davies, J., and Spies, T. D.: Phagocytic activity of neutrophils in anemias, J. Lab. & Clin. Med 30: 910, 1945.
24. Wiltshaw, E., and Moloney, W. C.: Histochemical and biochemical studies on leucocyte alkaline phosphatase activity, Blood 10: 1120, 1955.
25. Follete, J. H., Valentine, W. N., and Reynolds, J.: A comparison of human leukocyte phosphatase activity toward sodium β-glycerophosphate adenosine 5′-phosphate and glucose-1-phosphate, Blood 14: 415, 1959.
26. Valentine, W. N., and Beck, W. S.: Biochemical studies on leucocytes. I. Phosphatase activity in health, leucocytosis and myelocytic leucemia, J. Lab. & Clin. Med. 38: 39, 1951.
27. Valentine, W. N., Follete, J. H., Hardin, E. B., Beck, W. S., and Lawrence, J. S.: Studies on leukocyte alkaline phosphatase activity: relation to "stress" and pituitary adrenal activity, J. Lab. & Clin. Med. 44: 219, 1954.
28. Scrimshaw, N. S., Béhar, M., Arroyave, G., Viteri, F., and Tejada, C.: Characteristics of Kwashiorkor (Síndrome Pluricarencial de la Infancia), Fed. Proc. 15: 977, 1956.