10 JAN 1965

NAV1.954663.004

FINAL REPORT TO

OFFICE OF NAVAL RESEARCH

from

Division of Nutrition
Vanderbilt University
School of Medicine
Nashville, Tennessee

concerning

A Program of Research on Problems of Malnutrition in the Middle East and Africa (NAMRU-3 Region)

Supported by ONR Contract No. Nonr 2149

by

William J. Darby
Professor and Chairman, Department of Biochemistry
Director, Division of Nutrition, Vanderbilt University

William N. Pearson
Associate Professor of Biochemistry
Associate Director, Division of Nutrition
Vanderbilt University

In 1960 the current collaborative program between Vanderbilt University, the Staff of NAMRU-3, with the joint support of the ONR, was inaugurated. A nutritional biochemistry laboratory and a radioisotope unit were established, with planning consultants from Vanderbilt. These laboratories have since been expanded in parallel with improvements or expansion of related facilities, such as the clinical laboratory and ward at NAMRU-3, by a program of joint planning and utilization of resources and staff available to the Division of Nutrition at Vanderbilt University and NAMRU-3. Simultaneously, through the use of PL 480 funds for purposes of medical research in Egypt, collaborative programs have been established involving personnel at the National Institutes of Health (U.S.A.) and Egyptian medical scientists (at Munira Children's Hospital and the Institute of Nutrition), as well as senior members of the group at NAMRU and the Division of Nutrition of Vanderbilt University.

A major research facility has resulted, with its interest centered on nutritional problems and related conditions which are prevalent in the NAMRU region. These interests generally have been developed in the areas originally proposed, modified somewhat by the exigencies of research development and discovery and by available resources and personnel. This program will now be supported under an NIH grant, No. AM 08317-01, and have the continuing collaboration of scientists from Vanderbilt University and NAMRU-3.

Initially interest was directed toward the problem of anemia. Studies included:

(a) Investigation of the interrelationship of hookworm disease and iron deficiency anemia. This study showed a relationship between the severity of

anemia and magnitude of the hookworm infestation as quantitated by the number of parasites expelled after administration of an effective vermifuge.[1]

(b) Investigation of the absorption of radioactive iron by anemic and non-anemic subjects, of iron incorporated as an enrichment mixture in maize, and the availability of iron naturally occurring in food products (chicken, eggs, maize, beans, wheat)[2, 3]. These investigations as initially planned have been extended to study in greater detail the physiology of the metal in the plant when applied by injection into the growing stem as well as leaf application. Similar studies with zinc are now in progress.

Hoerman et al. described an abnormal fetal hemoglobin in thalassemia while working at NAMRU in 1960. The work initiated by Hoerman at NAMRU has been continued and expanded to include a clinical and biochemical evaluation of thalassemia as seen in Egypt. This work has been a collaborative study with Professor Diwany and his staff at the Munira Children's Hospital, Cairo University. In addition to confirming Hoerman's initial observations on the hemoglobinopathy associated with thalassemia this study has demonstrated the occurrence of low plasma zinc (and presumably zinc deficiency) in thalassemia. A manuscript describing these findings has been prepared and submitted for publication[4]. It is evident that thalassemia is not infrequent in this region and that it is an important clinical syndrome. On the other hand, there is no evidence that congenital hemoglobin abnormalities are a major consideration in the nutritionally related anemias. The significance of the apparent zinc deficiency in thalassemia is not completely clear, but it may be related in part to the growth retardation exhibited by children with this syndrome.

(c) Studies of the anemia associated with protein-calorie malnutrition in infants, both in Cairo and in Jerusalem, Jordan. Dr. Amin Majaj, in the latter city, had on insecure evidence previously reported the widespread occurrence of a macrocytic anemia among malnourished children treated on his pediatric service. Dr. James Dimming and Dr. Darby visited his service and were impressed with the probability that this anemia was macrocytic, that it was associated with a very low intake of protein, particularly of the sulfur-containing amino acids, and that it has certain attributes reminiscent of an experimentally induced anemia which occurs in Macaca mulatta when this animal is deprived of tocopherol. Detailed investigations of the subjects were planned and Dr. Dimming accepted an appointment from Vanderbilt University for the period from June, 1962 until March, 1963. In Jerusalem he organized a biochemical laboratory and provided leadership for the detailed investigation of this anemia. Fortunately, just as Dr. Dimming arrived in Jerusalem there was activated a National Institutes of Health grant awarded to Dr. Amin Majaj for study of protein-calorie malnutrition. The availability of these resources enabled the establishment of a nutrition research laboratory in the Augusta Victoria Hospital and the procurement of personnel. The latter were trained by Dr. Dimming and continue as members of a research team working with Dr. Majaj. At an appropriate stage the consultative assistance and counsel of Dr. Samir Assam, a hematologist trained with Dr. Finch's group in Seattle, Washington, and on the staff of the American University of Beirut, was enlisted for the work in Jerusalem and subsequently for review of the material in Cairo. Findings to date lead to the conclusions that: (a) associated with protein-calorie malnutrition in this region (Jordan, Lebanon, Egypt) there occurs in many

infants a macrocytic anemia with megaloblastic or erythroblastic marrow, associated with low levels of serum tocopherol and a creatinuria. This anemia has been found in Jordan to respond to administration of tocopherol, but not to administration of vitamin B_{12}. The latter is not surprising since the serum vitamin B_{12} levels are adequate in these infants. There is no evidence of ascorbic acid deficiency among these infants and initial cases studied in Jerusalem failed to respond to folic acid. Since then, a few cases have been observed exhibiting low folic acid levels in the serum and which subsequently responded to folic acid. (b) A rapid disappearance of creatinuria follows administration of tocopherol in these infants. (c) CoQ_4 likewise induces a hematologic and metabolic response which is similar to that observed following tocopherol administration. Studies of CoQ_{10} have to date given equivocal results[5-8].

These studies of anemia influenced Dr. Hasan Pharaon and others in the Ministry of Health in Jordan to initiate a year-round investigation of the occurrence of anemias in infants and preschool children at several representative areas in Jordan, further to define the nature of the anemia which occurs, and to obtain better evidence as to the prevalence of the macrocytic anemia described above. This study was a collaborative one between the ICNND (Interdepartmental Committee on Nutrition for National Development), ICNJ (Interdepartmental Committee on Nutrition for Jordan) and Vanderbilt University. Observations indicate that the occurrence of anemia in these malnourished infants may be seasonal and follows the period of the diarrheal disease.

(d) A correlative study of protein-calorie malnutrition syndrome in infants was made in collaboration with Professor Diwany and members of his staff at the Munira Children's Hospital, Cairo University. Detailed biochemical investigations have been made on 39 infants with typical kwashiorkor. The clinical and biochemical picture thus elaborated provides the fullest account to date of this syndrome in the Middle East[9]. The findings generally confirm those reported from other places in the world. New findings, however, include the observation of extremely low plasma zinc levels and elevated serum lactic dehydrogenase. The observation of exceedingly low levels of zinc in these infants who, in the post-kwashiorkor period, may be expected to exhibit continued growth retardation, coupled with observations cited below, lead to the suggestion that a state of chronic zinc deficiency initiated at the time of transition feeding may significantly influence rate of maturation and growth in regions commonly considered to be "protein deficient"[10]. In order further to test this hypothesis children in the preschool age group will be included in the proposed village study discussed subsequently. A more definitive description of the hematologic and biochemical levels of folic acid and iron in these cases of anemia and kwashiorkor is underway by Dr. Robert Weller. Results indicate that there are multiple deficiencies -- iron, folic acid, and vitamin B_{12} -- of varying degrees involved. However, there is some factor (or factors) other than these nutrients and protein which is required in order completely to restore the blood picture to normal.

(e) Interest in anemia provided an immediate opportunity to seek out and study in detail examples of the syndrome of dwarfism, infantilism and hypochromic microcytic anemia described earlier by Prasad from Shiraz[11]

Young men showing manifestation of this syndrome were found in villages in the vicinity of Cairo. Forty were studied in some detail and the following major points established[12-15]:

(1) Zinc deficiency exists in subjects with this syndrome. The evidence for the deficiency includes low levels of zinc in plasma, erythrocytes, and hair. Also a decreased urinary and dermal loss of zinc, a decreased 24-hour exchangeable pool of zinc and an accelerated plasma disappearance of radiozinc.

(2) In normal subjects there is a considerable loss of zinc in sweat which may amount to as much as 5 or so milligrams per day.

(3) Zinc deficiency frequently is associated with iron deficiency, but the presence of a deficiency of iron is not an obligatory component of zinc deficiency.

(4) Epidemiologic evidence has been accumulated in the oasis of Kharja[14] demonstrating the dissociation between zinc and iron deficiencies.

(5) Clay eating, parasitism, or blood loss are not essential to the occurrence of the zinc deficiency syndrome.

(6) This syndrome of dwarfism and infantilism appears to be associated with endocrinologic changes indicative of hypopituitarism[13, 16]. There is a carbohydrate dysfunction including a hypersensitivity to insulin. The pituitary ACTH reserve as measured by the SU4885 test is frequently impaired. Urinary pituitary gonadotropin is low. Bone age and height age are markedly retarded[13].

Administration of zinc to a selected number of cases has been followed by skeletal and body growth as revealed in increased stature, increased tibial length of X-ray, as well as the development of secondary sex characteristics including appearance of facial, axilliary and pubic hair, coupled with expected endocrinologic changes[16].

The evidence available at present from these extensive investigations indicate that a deficiency of zinc in the young child and adolescent is common in this region and is associated with growth retardation, infantilism and endocrinologic changes which include evidences of hypopituitarism, hypogonadism and a decreased production of adrenal steroids. Some of these changes are reversible by administration of zinc. The pathogenesis of zinc deficiency has not been entirely elucidated but initial investigations of the diet indicate a low intake (5 to 7 mg. per day) of zinc on the almost completely vegetable diet of these children. The possibility of unusual dermal losses of zinc as a result of climatic conditions, and excessive losses of the element along with iron as a result of blood loss of parasitism (hookworm and bilharziasis). All of these factors may be grafted upon the initial marked deficiency of the elements which occurs in many infants and young children at the transition period as noted above in relation to the studies on kwashiorkor.

It is evident that many of the above studies are concerned with an interrelationship between nutrition and parasitism. Another such investigation is that on patients with kala-azar in the region of Malakal in the Sudan. NAMRU-3 had at Malakal a laboratory for research on kala-azar. The nutriture of 25 patients with kala-azar and 35 controls without evidence of the disease was compared. Clinical and biochemical investigations were included. In all instances the subjects with kala-azar exhibited greater evidence of malnutrition in comparison with the controls[17].

In an effort better to define the host parasite metabolic relationship in bilharziasis, studies in the experimental disease have been instituted jointly by Drs. Schulert and Browne. These initial investigations are designed to

study the metabolism of inorganic portions of known effective therapeutic substances, specifically antimony. A remarkable series of observations has evolved as a consequence. The locale of the effect of the antimony-containing drug clearly is the ovum of the parasite[18]. This confirms an opinion held by some clinicians in Egypt, and clinical studies of this point are now underway. It is anticipated that similar investigations of metabolic interrelationships of host and parasite will be possible not only with therapeutically active materials but also with related nutrients. These studies may be expected to reveal findings of unusual interest.

In any program of this magnitude it is inevitable that alert investigators will accumulate several interesting investigative results which are outside of the main stream of study. One such resulted from the opportunity to study a case of hypogammaglobulinemia. In this investigation labeled gammaglobulin was administered and it was found that an unusual amount of the tagged gammaglobulin was excreted by the patient on the first day and, subsequently, there was an abnormal retention of the gamma globulin by the subject with the defect. A manuscript describing these experimental observations has been submitted for publication[19]. Other ancillary studies have involved studies of chronic lead intoxication on I^{131} uptake of rats[20] and clinical studies of bilharzia[21], pityriasis alba[22], and Brucellosis[23].

(7) Parallel investigations at Vanderbilt University have included studies in the rat of iron absorption which lead to the conclusion that absorption of iron at the intestinal mucosal level in this species is independent of body stores of this nutrient, but that the rate of transport of the nutrient from mucosal cells is influenced by body stores. In the instance of zinc there appears to be

no such regulatory mechanism operating[24]

Studies of the interrelationships of tocopherol and selenium have been made in animals, first in an effort to ascertain whether it is possible in the rat to induce a tocopherol or tocopherol-selenium deficiency anemia. Efforts to produce such an anemia in the rat have been unsuccessful. However, evidence has been accumulated which further support the concept that these two nutrients are intimately interlinked in nutrition, that selenium exerts a tocopherol-sparing effect but that tocopherol exerts little, if any, such selenium-sparing effect. An interesting additional observation has been that there is a remarkable sex specificity in relation to production of selenium-tocopherol deficiency -- males being much more susceptible to depletion than females. Somewhat unexpectedly this susceptibility of the male rat to the deficiency seems to be greatly reduced by administration of estrogens[25].

Studies on zinc deficiency in the albino rat have been carried out in an effort better to define the biochemical lesion present in this deficiency state. It has been possible to produce a zinc-free casein which used as the protein of the basal zinc-free diet and fed to rats results in the cessation of growth within a two-week period. Studies in the rat have indicated that this growth arrest is not influenced by growth hormone. However, these studies have revealed that in the rat zinc deficiency results and an abnormality of carbohydrate metabolism, which finding is reminiscent of observations made in Cairo in zinc deficient dwarfs in which evidence of carbohydrate defect has been noted. These results currently are being expanded in the rat and human and efforts to reproduce them in the monkey are underway.

BIBLIOGRAPHY

1. Farid, Z., and Miale, A., Jr. Treatment of Hookworm Infection in Egypt with Bephenium Hydroxynaphthoate and the Relationship Between Iron Deficiency Anemia and Intensity of Infection. Am. J. Trop. Med. & Hygiene, 11: 497-505 (1962).

2. Miale, A., Jr., Prasad, A., Farid, Z., Schulert, A., and Sandstead, H. Radioiron Absorption in Anemia Patients with Parasitic Infections in Egypt (Abstract). Proc. Middle East Medical Assembly. Beirut, Lebanon. 1962.

3. Farid, Z., and Miale, A., Jr. Comment in report entitled "Anemia in East Africa, Medicine and Surgery in the Commonwealth", Brit. Med. J., 1: 549 (1962).

4. Prasad, A. S., Diwany, M., Gabr, M., Sandstead, H. H., Mokhtar, N., and El Hefny, A. Biochemical Studies in Thalassemia. Submitted for publication.

5. Majaj, A. S., Dinning, J. S., Azzam, S. A., and Darby, W. J. Vitamin E Responsive Megaloblastic Anemia in Infants with Protein-Calorie Malnutrition. Am. J. Clin. Nutrition, 12: 374 (1963).

6. Dinning, J. S., Majaj, A., Azzam, S., and Darby, W. J. Vitamin E Responsive Megaloblastic Anemia in Children. Federation Proc., 22: 550 (1963).

7. Dinning, J. S., Majaj, A. S., Azzam, S. A., Darby, W. J., Shunk, C. H., and Folkers, K. Response of Macrocytic Anemia in Children to the Coenzyme Q_4-Chromanol. Am. J. Clin. Nutrition, 13: 169 (1963).

8. Majaj, A., Dinning, J., Folkers, K., and Darby, W. J. Unpublished Observations.

8a. Interdepartmental Committee on Nutrition for National Development and Interdepartmental Committee on Nutrition for Jordan. Hashemite Kingdom of Jordan: Nutrition Survey on Infants and PreSchool Children in Jordan. November 1962-October 1963. In preparation.

9. Sandstead, H. H., Gabr, M., Azzam, S. A., Shukry, A. S., Weiler, R., Mokhtar, N., Prasad, A. S., Mohy el Din, E., El Hefney, A., and Darby, W. J. Kwashiorkor in Egypt. I. Hematologic Aspects: The Occurrence of a Megaloblastic Anemia Associated with Low Serum Vitamin E and a Wide Range of Serum B_{12}. Am. J. Clin. Nutrition, in press.

10. Sandstead, H. H., Shukry, A. S., Prasad, A. S., Gabr, M. K., El Hefney, A., Mokhtar, N., and Darby, W. J. Kwashiorkor in Egypt. II. Clinical and Biochemical Studies, with Special Reference to Plasma Zinc and Serum Lactic Dehydrogenase. Am. J. Clin. Nutrition, in press.

11. Prasad, A. S., Halstad, J. A., and Nadimi, M. Syndrome of Iron Deficiency Anemia, Hepatosplenomegaly, Hypogonadism, Dwarfism and Geophagia. Am. J. Med., 31: 532-546 (1961).

12. Prasad, A., Miale, A., Jr., Farid, Z., Schulert, A., and Sandstead, H. H. Zinc Metabolism in Patients with the Syndrome of Iron Deficiency Anemia, Hepatosplenomegaly, Dwarfism and Hypogonadism (Abstract), Proc. Middle East Medical Assembly, Beirut, Lebanon, 1962.

13. Prasad, A. S., Miale, A., Jr., Farid, Z., Sandstead, H. H., Schulert, A. R., and Darby, W. J. Biochemical Studies on Dwarfism, Hypogonadism and Anemia. Arch. Int. Med., 111: 407 (1963).

14. Prasad, A. S., Miale, A., Jr., Farid, Z., Sandstead, H. H., and Schulert, A. R. Zinc Metabolism in Patients with the Syndrome of Iron Deficiency Anemia, Hepatosplenomegaly, Dwarfism, and Hypogonadism. J. Lab. Clin. Med., 61: 537 (1963).

15. Prasad, A. S., Schulert, A. R., Miale, A., Jr., Farid, Z., and Sandstead, H. H. Zinc and Iron Deficiencies in Male Subjects with Dwarfism and Hypogonadism but without Ancylostomiasis, Schistosomiasis or Severe Anemia. Am. J. Clin. Nutrition, 12: 437 (1963).

16. Sandstead, H. H., Prasad, A. S., Miale, A., Jr., Schulert, A. R., Farid, Z., Bassaly, M., and Darby, W. J. Endocrine Abnormalities Associated with Human Zinc Deficiency. In preparation.

17. Sandstead, H. H., Schulert, A. R., Darby, W. J., Rohrs, L., and with technical assistance of J. Robbinson. Kala-azar in the Sudan: Nutritional and Biochemical Observations. In preparation.

18. Browne, H., and Schulert, A. R. Disposition of Sb^{124}-Labeled Astiban in Animals with Bilharziasis. Federation Proc., 22: 666 (1963).

19. Prasad, A. S., Aboud, M. A., Salawi, A., and Schulert, A. R. Hypogammaglobulinemia: Iodinated Gamma Globulin Studies. Submitted for publication.

20. Sandstead, H. H., and Galloway, R. The Effect of Chronic Lead Intoxication on I^{131} Uptake and Conversion Ratio in Rats. In preparation.

21. Farid, Z., Prasad, A. S., Schulert, A. R., Sandstead, H. H., and E. Rooby, A. S. Bilharzial Splenomegaly. Arch. Int. Med., in press.

22. Bassaly, M., Miale, A., Jr., and Prasad, A. S. Studies on Pityriasis Alba, A Common Facial Skin Lesion in Egyptian Children. In preparation.

23. Farid, Z., Miale, A., Jr., Omar, M.S., and Van Peenen, P.F.D. Antibiotic Treatment of Acute Brucellosis Caused by Brucella melitensis. J. Trop. Med. & Hygiene, 64: 157-163 (1961).

24. Pearson, W.N. Unpublished investigations.

25. Molnar, G. Biochemical and Nutritional Interrelationships Between Vitamin E and Selenium in the Albino Rat. M.S. Thesis, in preparation.