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STUDIES ON PROTEIN-CALORIE MALNUTRITION IN EGYPT.(U)
MAR 77 D FLEITA, S EL MAWAZINY

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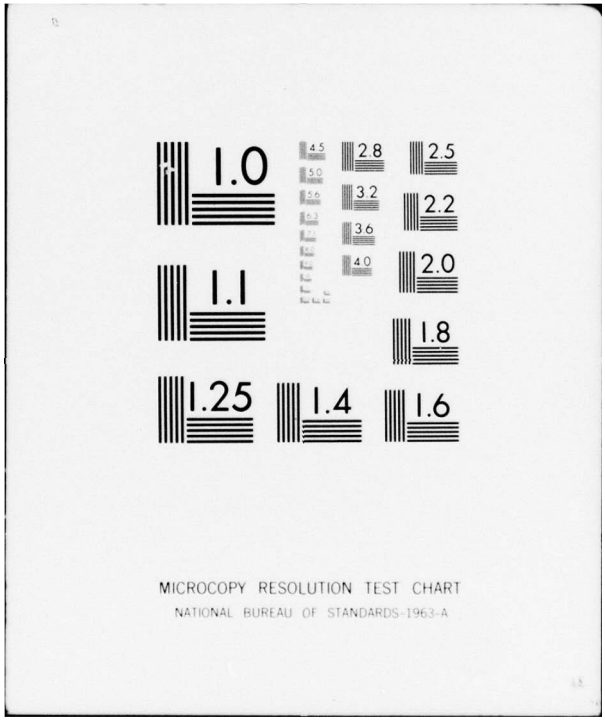
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THE AMERICAN UNIVERSITY IN CAIRO
Department of Materials Engineering and Physical Sciences ✓

⑥ STUDIES ON PROTEIN-CALORIE MALNUTRITION IN EGYPT.

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⑨ CUMULATIVE PROGRESS REPORT.
(September 1971 - March 1977)
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Contract

⑮ : N00014-71-C-0437 *New*

Principal Investigators: Dr. Daisy Fleita, Associate Professor
Dr. Sheila El Mawaziny, Associate Professor

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INTRODUCTION

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The validity and usefulness of many indices used for delineating forms and grades of protein-calorie malnutrition have been questioned by a number of researchers. This is not surprising, since the tests reported were carried out by different investigators and at times in different localities by different laboratory techniques and even on different clinical variants of the syndrome.

→ Protein-calorie malnutrition ^(PCM) is the commonest form of nutritional deficiency found in infants and children in poor sectors of the Egyptian population. Developing simple criteria for the early diagnosis of the disease in Egypt could make a significant contribution to public health here. Furthermore, the presence of numerous cases in Egypt makes possible statistically significant laboratory analysis of various aspects of the syndrome. For these reasons this project has for the past five and one-half years carried out an extensive laboratory survey of PCM, in coordination with relevant supporting clinical and field activities, including: (1)

→ (Cont on p. 2)

The technical results of the project have been published in the form of 21 papers, copies of which are attached. From its inception until her death in August 1976, the project was inspired and directed by Dr. Akila Said, Professor of Chemistry at the American University in Cairo. The current investigators agreed to continue the project through to its completion in August 1977. In view of the change in principal investigators, they felt that the Office of Naval Research should have a comprehensive cumulative progress report on work to date. It is their belief that this is a most worthwhile project that has yielded reliable results on a number of important aspects of the PCM syndrome.

(cont'd p. 1)

From beginning the project was approached by:

- a) Studying the various clinical forms and variants of the syndrome; (2)
- b) Investigating the various biochemical derangements that might be associated with these clinical types; (3)
- c) Follow-up of the biochemical changes that might happen during recovery or progress of cases and utilization of the results in diagnosis, therapy and prognosis; (4)
- d) Finding out the high risk factors of the syndrome by studying the social background of patients; and (5)
- e) Whenever possible, some studies on experimental animals. especially when the investigations cannot be applied to human beings.

RESEARCH COMPLETED
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Despite the difficulties mentioned later and the passing of Dr. Akila Said, who conceived and directed the project in the first five years, the investigators believe that they have developed reliable criteria of objectively diagnosing PCM cases. Since September 1, 1971 1420 PCM cases (700 in the first three years and 720 from September 1974 till now) were clinically studied, categorized and investigated. Parallel controls were similarly investigated.

MATERIAL OF STUDY

The material of study included infants and young children suffering from PCM as well as parallel normal control patients. All patients were chosen from the out-patient clinic at the Mounira Children's Hospital, Cairo University. The diseased subjects comprised only those suffering exclusively from PCM while the control subjects were selected from disease-free patients at the hospital. All patients, diseased and controls, were hospitalized for one night in order to obtain samples of fasting venous blood, 24 hour urine and/or morning saliva, depending on the research being undertaken. Patients for whom treatment was prescribed received follow-up analysis at the out-patient clinic.

Samples used for the investigations listed below were multiple samples i.e. blood and urine, blood and saliva, or urine and saliva, and tests were carried out in parallel as far as possible on the same samples.

CLINICAL CATEGORIZATION

In the first report and publications cases were categorized into:

- a) Kwashiorkor (mild, moderate and severe)
- b) Nutritional oedema
- c) Marasmus (first, second and third grade)
- d) Disease-free patients

At present the trend is to classify this spectrum of disease into two forms:

(1) Oedematous, and (2) Non-oedematous. The first form includes all cases of PCM presented with oedema whether of the pure KWO type or of the marasmic KWO variant and the second includes all forms of marasmus. All cases with oedema are suffering from hypoproteinemia whereas in non-oedematous cases, the serum proteins are within normal or just below the lowest normal level.

This classification has been found easier in interpretation of findings and of practical value in diagnosis, management and prognosis.

I. BIOCHEMICAL TESTS

A. SERUM, URINE AND RBC'S

1. Protein Metabolism: Total proteins and the five major fractions as well as the individual protein components were evaluated in serum, urine and plasma (1,2,3,4).

a) Urinary proteins immunophoretic studies (1) proved to be of certain diagnostic and prognostic value. In oedematous forms (KWO and marasmic

KW0) of the disease the pattern showed progressive changes with the advancement of the disease revealing albumin in higher concentrations than normal in mild cases associated with a number of other protein components in severe cases. In non-oedematous forms (marasmus) the urinary proteins immuno-pattern was somewhat consistent, yet appearance of an increased number of protein components was usually indicative of the advancement of the disease. Normalization of the urinary protein immuno-pattern proved to be a good sign of response to treatment and usually accompanied clinical cure.

b) Simple micro-agar gel electrophoresis of protein fractions showed that proteinuria in mild cases affects albumin only, while in severe cases of KW0, third grade marasmus and marasmic KW0, the proteinuria affects albumin and all other serum fractions (2,3).

c) Serum and urine total proteins: Serum total proteins and all of its fractions (Alb, α_1 G, α_2 G, B-G, α -G) showed a significant decrease but no specific pattern for any disease entity or its stage could be found (2).

The partial loss of all serum proteins including albumin and globulins to urine, in most cases particularly the severe ones (2), seems to be a diagnostic index indicating the severity of the disease, as well as being a bad prognostic sign as cases showing relatively high proteinuria were usually fatal.

On the other hand, urinary total proteins increased in different degrees with the different forms of PCM. Increased proteinuria in PCM seems to be an accompanying feature of the disease and its aggravation goes parallel with increased clinical severity of the disease.

2. Amino Acids Metabolism:

Amino acids, total and individuals were determined in serum, urine and RBC's (12, 18 20).

a) Serum and urine amino acid pattern and serum creatinine levels were studied. Hypoaminoacidemia affecting both essential and non-essential amino acids was a general feature, being associated with hypoaminoaciduria, except in mild KWO.

These changes in serum and urine amino acid pattern add support to the suggestion that nutritional oedema is a separate entity of PCM. In addition, a serum valine/creatinine ratio, different from other variants, was found to be a better index to our community than other ratios because it gave a clear distinction between different grades and types of PCM. So it seems to be a useful adjunct to delineate the various grades of the syndrome.

b) The efficiency of intestinal absorption was tested as it is postulated to be of importance in visualizing derangement of amino acid metabolism in PCM. After oral administration of 3 amino acids (lysine, tryptophan, cysteine), an elevation was obtained in the level of total amino nitrogen in plasma reaching its maximum at the first or second

hour after the dose, thereafter the level declined in a more or less irregular manner.

The rate of absorption of lysine, tryptophan and cysteine (as calculated from an absorption index) was noticed to be affected by the derangements caused by PCM. Retardation in absorption may be attributed to several factors operating in the process of intestinal absorption and transfer, and hence in utilization.

c) Red blood cells amino acid pattern in PCM was investigated. The results revealed that there is a drop in total amino acids in RBC's of KWO patients comparable to that reported in plasma which was attributed to dietary protein insufficiency. Such similarities in the general patterns of serum and RBC's may indicate an equilibrium between extra and intra cellular components.

d) Following oral administration of lysine, tryptophan and cysteine a rise in RBC's total amino acids occurred similar to that in plasma. The calculated transport index ⁽²⁰⁾ indicated that all categories of the disease showed a retarded rate of transport of amino acids to the red blood cells, this may be due to shift of transport mechanism from the active type to simple exchange or to abnormalities in the red cell membrane. This study needs further investigation to be clarified.

It may be concluded that the RBC's amino acids are also affected by protein-calorie malnutrition in a similar way to those of plasma. But to utilise RBC's as representative of other tissue cells needs further study.

3. Enzyme Activities:

We have studied the pattern of enzymes in serum, urine, whole blood packed cells and RBC's. It is evident from the study that the type and extent of the change in serum enzyme activities of transaminases (GOT, GPT) and phosphatases (acid, alkaline) varied fairly consistently from one type of PCM to another as well as among the different grades of the same variety, a finding which might help in diagnosis and prognosis ⁽⁷⁾. Such a conclusion is supported by the fact that the liver in KWO is invariably involved but cirrhotic changes are very rare in Egypt.

Cholinesterases in serum and liver which can be used to foretell the state of liver functions were examined ⁽⁹⁾. The results revealed that in oedematous forms there was a decrease in serum cholinesterase activity concomitant with a parallel decrease in liver cholinesterase. The extent of decrease was found to be parallel to liver damage.

On the other hand, non-oedematous forms of PCM showed no significant change in serum and liver cholinesterase.

So the changes in serum and liver cholinesterase seem to serve as indices of the extent of liver involvement. This is in addition to their diagnostic and prognostic values.

Amylase activity in serum and urine together with blood sugar level were estimated ⁽¹⁹⁾. The values obtained for serum showed decreased levels of amylase activity in early and severe stages of KWO while increased amylase activity was observed in marasmus. These findings with

the non-significant correlation between serum amylase activity and blood sugar level in diseased cases indicate deranged function of the pancreas. Urine amylase activity was high in all groups of PCM, which might be a consequence of renal derangement leading to amylasuria. Such amylasuria disappeared with disappearance of proteinuria on clinical recovery, indicating the transient nature of renal derangement. The activity of the enzymes, phosphatases (acid and alkaline) and transaminases (GOT, GPT) were investigated in the urine of PCM patients (16). Enzymuria was observed in PCM, especially severe cases. Such enzymuria did not seem to be a simple reflection of the increased serum values and suggest renal involvement.

Following the results obtained for the enzymatic activity in urine, it was concluded that the urine enzyme analysis may reflect the extent and state of renal pathology among different forms of PCM.

4. Electrolytes

Plasma and RBC's electrolyte content were analysed (21). Hyponatremia was dominant in the different categories and grades of the disease. The decrement was more aggravated in KWO and was attributed to the associated gastrointestinal loss and oedema. On the other hand RBC's sodium content was little affected.

The difference between the level of potassium in KWO and marasmus was clearer in RBC's than in serum. Our RBC's results support the view that RBC's behave similarly to muscles in being depleted from potassium, particularly in severe states. Accordingly it can be suggested that the

relatively easy analysis of minerals in RBC's can reflect similar changes in tissues of PCM subjects and make muscle diopsy unnecessary.

5. Haematological Studies:

Haematological investigation of the types of anemia associated with the different forms of protein energy malnutrition has been carried out (10,11,15). The work included the determination of blood hemoglobin, hematocrite, total erythrocyte count and other red cell indices (MCV, MCH and MCHC) in venous blood samples.

Results have shown that the anemia associated with moderate KWO cases was predominantly of the hypochromic type (83.3%), being normocytic in 50% of cases and macrocytic in 33.3% of cases.

In severe KWO, normocytic anemia was detected in 42% and microcytic in 19.2% of the investigated cases. As microcytosis was observed only among a few particularly severe KWO cases, this may suggest that iron deficiency anemia is not the type of anemia encountered among KWO cases and in turn is not the sole effective factor in its causation. This may be supported by the low serum iron level with high transferrin saturation level.

Macrocytosis was detected only among 38% of severe KWO cases indicating diminished erythroid activity in the bone marrow, leading to improper functions and eventually the release of the abnormally macrocytic red cells into the circulation. This may be supported by the low reticulocyte count observed in severe KWO cases.

In marasmus, normocytic anemia was revealed in 74% of the 2nd grade cases and in 70% of 3rd grade cases. Hypochromia was only found in 42% of marasmic cases.

Hypochromia is therefore more predominant in KWO compared to marasmic cases. The latter is due to insufficiency of globin, iron or both, together with such other participating factors as Vitamin B₁₂ and folic acid. In spite of the relatively high percent of transferrin saturation with iron in KWO, it seems that deficiency of proteins is the limiting factor in hemoglobin synthesis. On the other hand in marasmus although protein and iron are both deficient yet they are available in equivalent amounts to enable the hemoglobin synthesis to proceed.

B - SALIVA

Collection of Saliva:

After clearing the buccal cavity on a fasting morning a whole saliva sample is collected during an interval of 35 minutes. Sterilized cotton plugs are introduced and left in the buccal cavity using sterilized forceps. When the cotton plugs become soaked with saliva, (the samples collected during the first 5 minutes are rejected, those during the next 30 minutes are collected) samples are collected in Wassermann tubes placing the cotton plugs in sterile plastic syringes and repressing them to deliver their contents. By this way 4-5 ml of saliva can be obtained from every individual. Thereafter samples are centrifuged for 15 min. at 3,000 rpm and supernatant fluid is collected in another tube.

Total protein and its components, amino acids, minerals, enzymes, (amylase, GOT, GPT and phosphatases), glycoproteins, total reducing sugar and total nitrogen were investigated in 98 controls as well as 245 PCM cases.

The data obtained for saliva whether for controls or PCM are the first to be obtained in Egypt. In addition the results obtained revealed that the changes in total amino acids, proteins, electrolytes and amylase activity in saliva are significant and may help in diagnosis and treatment of PCM disease.

The findings regarding immunoglobulins in saliva until now represent similar changes that are encountered in the sera of these patients. However, they cannot explain the low immunity met with in PCM cases, but it enables one to dispense with serum sampling.

Immunoglobulins studied namely IgA, IgM, IgG and IgE are not specific for the mucous membranes. It is intended to estimate secretory IgA (specifically for the mucous membranes) of eyes, mouth, nose, trachea, bronchi and gastro intestinal tract. Estimation of this specific IgA would provide us with a more exact reflection of the state of the mucous membranes which are the first line of defence in immunity, and certainly would explain why these patients are vulnerable to infection.

II SOCIO-ECONOMIC STUDIES

This investigation was planned to throw some light on the high risk factors related to PCM among Egyptian infants. The study included PCM infants and their families from those seeking medical care at the Pediatric Department (Mounira Children's Hospital) Faculty of Medicine, Cairo University. A number of socio-economic medical parameters have been investigated (6).

The material of study included 232 PCM cases (112 females and 120 males) whose age ranged from 3 - 30 months, the results revealed the following:

1. The disease affects infants of both sexes in almost the same ratio.
2. The incidence of the disease is low at ages below six month but some-what high among infants of 18 - 21 months old.
3. The number of cases in Egypt showing the severe stages of each disease entity exceeds that of early or moderate stages of the same disease.
4. The frequency of different seasonal disease affecting the infants prior to the appearance of PCM as indicated from the clinical history of the families of low social-classes.
5. High incidence among the first three offsprings. Malnutrition was also found to affect infants of the order from the fourth to ninth but the percentage incidence of the disease showed progressive decrease with increasing order of the infant among other family siblings.

6. Malnutrition affects infants growth.
7. Correlation between the mortality rate in infants and young children and the different forms and grades of PCM: marasmic kwashiorkor cases seem to be the severest form of PCM, and nutritional oedema is the mildest one.

It was concluded that the high frequency of malnutrition among Egyptian infants and young children of poor families might be one of the major factors responsible for the high infant mortality rate.

III STUDIES ON SPERMATOGENESIS IN RAT TESTES

In this work 60 male albino rats were subjected to a feeding experiment, aimed to study the influence of protein free and reduced diets on normal functions. The nature and severity of these effects depend on the duration of the deficiency state. Histological examination of rat testes, revealed that the spermatogenesis is arrested at spermatogonium stage in rats fed on protein free diet, and at primary and secondary spermatocyte stage in rats fed on normal but reduced diet (17).

RESEARCH CONTINUED

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Studies are being carried out on patients with protein energy malnutrition to determine some immunological aspects of their defense mechanisms.

1. Cell mediated immunity (Tuberculin test, dinitrochlorobenzene test (DNCB)).

2. Leucocytic function (leucocytic enzymes, T.B. lymphocytes, phagocytosis and bactericidal power).
3. Humoral immunity (immuno globulins essay in serum namely IgA, IgM, IgG and IgE).
4. Blood picture.
5. Field survey to study the social background and the "high risk factors".

We have just begun the work at Basaisa Village in which we will study the factors effecting the diet and health of approximately 250 villagers. The village is 100 Km from Cairo and although it is fairly close to a large town it is relatively isolated. Each villager will have a thorough physical examination and data will be collected relating to facilities, diet, income and other factors which may contribute to nutritional imbalances and/or ill-health. The project doctors will treat any villagers for minor diseases. If the project is extended it is intended to carry out a comparative study on a low-income group of Cairenes.

Some parts of the "research continued" were not mentioned in any previous proposal and have been continued because the personnel carrying out these determinations have been already trained on the techniques during April and May, 1976 and the chemicals were purchased. In addition these determinations seem to be important in clarifying the decreased immunity of PCM patients. The results of these investigations will be included in the next progress report in July 1977.

INCOMPLETE WORK
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In spite of the difficulties encountered at the beginning of the last year, we tried to fulfill most of the objectives mentioned in the project proposals of September 1971, 1974 and 1976. However because of the limited period left before termination we are unable to cover the following parts:

- 1) Iso enzymes pattern. Unfortunately we are unable to undertake research projected on iso enzymes, because the equipment and materials needed are not available at the moment. However one of our Ph.D. biochemists working in the project has gone to a research laboratory in Denmark for 6 month for training on the recent techniques used for the iso-enzymes investigation and he will be back by September 1977.
- 2) Trace elements: This analysis was delayed originally for lack of an atomic absorption spectrophotometer. Though finally purchased in August 1976, startup has been delayed by difficulties in installation and calibration. We will start this research as soon as possible and complete as much as time allows.
- 3) Chemotaxis and opsonization: They were mentioned in Dr. Said's proposal of September 1, 1976 but it was difficult for the present investigators to start this work because of lack of chambers, materials and a well trained biochemist.
- 4) Follow-up of recovered cases: There is follow-up only in the hospital during treatment and immediately after recovery.

- 5) Computerizing the data obtained: The individual responsible for computerizing project data left Egypt to accept a fulltime position in Iraq. We believe that this aspect of the project is important and shall try to carry out this work.

ADMINISTRATIVE COMMENTS

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CHEMICALS

Some of the project work called for very specific chemicals and although we searched extensively on the local market they were unavailable. For example spontaneous rosette assays were going to be carried out by the method of Pang, Baguiley and Wilson (1974) but we had to replace it by the method described by David et al (1974).

EQUIPMENT

In some cases pieces of equipment require an expert to get them established and in good working order. For a long time we could not find someone to set up the atomic absorption spectrophotometer, the supplier being sick or out of the country. We have finally found a qualified engineer through the supplier. We feel that in the future if the project is extended and if another major piece of equipment is purchased it may be an excellent idea to send one of the project's personnel for training abroad.

Since last September we have tried to ensure that all equipment was good working order. Some items had to be repaired. Although there are still one or two items which, for one reason or another, are not

in good shape, most of the items listed below are in very good or excellent condition. Our most valuable pieces of equipment are:

1. Mettler Balance
1. Beckman Spectrophotometer
- 1 Bio-Dynamics DIGITEX multi-wavelength photometer
- 1 Centrifuge (Christ Hearacus Christ) with a cooling system
- 2 Air Compressors
- 1 Freezer
- 2 Microscopes
- 1 Shandon Atomic Absorption Spectrophotometer
- 1 Manual (Carl Zeiss) Spectrophotometer

SPACE

The laboratory is situated in a five room flat, 10 minutes walking distance from the American University in Cairo. Three rooms are used purely as labs, one room for lab and office space and one room for an office and small library. There is also an equipped kitchen.

PERSONNEL

The expansion of the project to include immunology calls for highly qualified personnel. At the beginning of last year we found some difficulty in starting some aspects of the work especially the immunology due to lack of qualified personnel. We were finally successful in obtaining the services of a very highly qualified individual, Dr. Esmat Megala Ekladiose who began working with the project.

At present, March 1977, we have the following personnel working at the project mostly on a part-time basis.

The two principal investigators Dr. Sheila Mawaziny, Dr. Daisy Fleita

One Senior paediatrician (M.S.) Dr. Rashed Sakr

Two paediatricians (M.D.) Dr. Mohamed Abdel-Khalek, Dr. Samiha Samuel

One Senior immunologist (M.D.) Dr. Esmat Eladiose

Three biochemists (Ph.D.) Dr. Mohamed Bahgat, Dr. Ahmed Ibrahim
Dr. Maher Abdien

One chemist (Ph.D.) Dr. Hakim Grace

One fulltime Chemist (B.Sc)

Two biochemists (B.Sc. and M.Sc.)

Two technicians (B.Sc)

One fulltime Janitor

One fulltime Driver

One Secretary

One Administrative Assistant

THE AMERICAN UNIVERSITY IN CAIRO
DEPARTMENT OF MATERIALS ENGINEERING AND PHYSICAL SCIENCES
OFFICE OF NAVAL RESEARCH PROJECT N00014-71-C-0437

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