Biochemical measurements in the assessment of the protein nutrition status

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SUMMARY

The author discusses the problems encountered in the appraisal of the protein calorie nutrition status. The biochemical tests discussed are total serum protein, serum albumin, serum amino acid ratio, urinary creatinine-height index, urinary hydroxyproline index, urinary urea nitrogen/creatinine ratio, and the urinary inorganic sulphate sulphur/creatinine ratio.

It is concluded that for the present time and probably into the near future the hope of assessing the PCM status in communities lies in the integration of dietary, anthropométric and biochemical tests.

INTRODUCTION

Numerous nutrition surveys have been conducted around the world and within the framework of most of these surveys an effort was made to establish the protein or protein-calorie nutrition status of the population group being surveyed. The methods employed usually consisted of clinical, anthropometric, dietary, and biochemical assessments. However, by using these various disciplines no good system has been devised to integrate the data from the various disciplines into an effective "system" or "profile" to determine the protein-calorie nutrition status of the population groups being studied.

During the past decade many scientists and nutrition experts have been concerned with the problem of insufficient protein in the world. However, recently it has been noted that the major deficiency in diets of the poor in developing coun-

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Recibido: 21-10-1971.

tries is also that of calories. An increased evidence indicates that the latter of the two deficiencies is on the upsurge. This deficiency of calories greatly affects the picture of protein deficiency and hence the term protein-calorie malnutrition (PCM) (1) was devised. This term is being used in this report to cover mild to severe cases and includes both kwashiorkor and marasmus.

The author wishes to discuss various aspects of clinical signs, anthropometric measurements, dietary data, and biochemical measurements and indicate the problems confronting the nutritionist today with the assessment of the protein-calorie nutrition status of population groups. However, since all aspects of both clinical signs and anthropometry are expertly covered in the monograph by Jellife (2) they will only be briefly discussed here. Also the reader is refered to the FAO monograph (4) by Emma Reh for guidance in conductiong and analyzing the results from dietary surveys. A similar article has been written by the Committee on Procedures for Apprisal of Protein-Calorie Malnutrition of the International Union of Nutritional Sciences (5). The present article gives more of the authors own views and a more complete bibliography.

Clinical Assessment

First, clinical signs while being useful have certain limitations. The clinical signs usually seen in kwashiorkor such as oedema, despigmentation of the hair, muscle wasting, easy pluckability of the hair, thin sparse hair, straight hair, diffuse pigmentation of the skin, psychomotor changes, moon-face, hepatomegaly, and flack paint-dermatitis and adipose tissue wasting in marasmus (2) (3) are usually seen in children already admitted to the hospital and are rarely encountered in the field. The socalled "pre-kwashiorkor", "sub-clinical kwashiorkor", or "early nutritional marasmus" is usually seen in field surveys, and as can be imagined, clinical sings of these mild to moderate cases are difficult to recognize.

Also, clinical signs are difficult to standardize and many times different results from different investigators on the same population have been noted.

However, it should be pointed out that the detection of clinical signs can be carried out without costly equipment and one examiner can examine many children in a short period of time. Also clinical signs are more useful in areas where the predominant form of PCM is kwashiorkor.

Certainly, clinical signs should be recorded in any nutrition survey because they may greatly aid the investigator to determine the occurrence of the two severe syndromes (kwashiorkor and marasmus) in any given population. But, their limitations have to be taken into account.

Anthropometric Measurements

Nutritional anthropometry is used to measure variations of physical dimensions of the body at different ages in relation to the nutritional status. Since growth retardation with the resulting body disproportion is one of the first indicators of mild PCM nutritional anthropometry can be one of the most valuable objective measurements in assessing the protein-calorie nutrition status.

The main measurements used are weight, height (length), arm circunference, triceps skinfold, and head and chest circunference. These measurements are then related to either international or local standars from the same measurements; usually international standards.

One problem encountered in anthropometry is that weight, height, arm circunference and triceps skinfold are dependent upon knowing the precise age. Since in many developing countries the exact ages are not recorded this can be a major problem (Africa mainly). However, the chest-head ratio, weight-for-height and weight-for-head circunference may be used when ages are not known (2).

Also, anthropometry, as clinical signs, is easy to conduct in the field and many children can be examined in one day with a small staff and little equipment. In populations where marasmus is the predominant form of PCM encountered anthropometry may be the only way to assess the nutritional status.

It should be noted however, that low or abnormal anthropometric measurements simply indicated a reduced growth rate and do not necessarily indicated any epecific nutritional deficiency. But when used with other nutritional measurements such as clinical signs, dietary data, or biochemical measurements they can give invaluable information in helping to evaluate the nutrition status.

Dietary Status

Dietary data is probably the most widely used of nutritional indicators in surveys but the worst analyzed and mos underestimated. It is amazing to see that with the numerous nutrition surveys conducted around the world there is little precise data on the actual intake of protein and calories of pre-school age children. Most of the data on the diets of preschool age children is taken from information that has been obtained through conventional weighing of the entire family diet and use of an arbitrary coefficient to compute the diet of the pre-school age child.

Since the science of nutrition has advanced to more exactness totay it is possible to define the requirements of both protein and calories for different age groups. Therefore, the exact dietary data from the pre-school age child could be extremely useful in helping to assess the nutrition status of children in this age group.

Good dietary data does not only indicate the presence (only gross unless individual data is obtained) of protein and calories in relation to requirements but also has the advantage of being table to indicate the habits of the children expressing the different nutritional deficiencies.

Also dietary data is extremely helpful in interpreting the clinical and anthropometric measurements of wich most are nonspecific. Dietary data if properly collected and used could be the most valuable parameter in helping to assess the PCM status of different population groups.

Biochemical Measurements

Due to the non-specificity of both clinical and anthropometric measurements the physicians have turned to the biochemist to help solve the problem of the assessment of the PCM status. Unfortunately, even after around five years of intensive research by many excellent investigators no good single biochemical test has been developed for this assessment that is useful in all parts of the world. This is amazing since biochemists have already developed excellent procedures to evaluated the nutrition status in regard to some other nutrients such as riboflavin or vitamin C.

The assessment of the protein nutrition status is more complicated for several reasons. First, the biochemist is not only confronted with the problems of a low protein intake but also with the interplay of calories. Secondly, other superimposed factors such as infections and worm infestations seem to also play a major role. Finally, deficiencies of ther vitamins or minerals may also have an effect.

The following biochemical tests will be discussed with both their merits and limitations. They are a) total serum protein, b) serum albumin, c) serum amino acid ratio, d) urinary creatinine-height index, e) urinary hydroxyproline index, f) urinary urea nitrogen/creatinine ratio, and g) urinary inorganic sulphate sulphur/creatinine ratio their constraints.

Total Serum Protein

Probably no other biochemical test has been so widely used around the world with such little success as the estimation of the total serum protein. The measurements of the total serum protein level as an index of protein nutriture in human populations has been carried out with a devotion that approaches fetishm. In most nutrition surveys where any biochemistry was done the total serum protein was determined. However, after many years of testing it is the opinion of most authors that there is little information to be gained from this determination (2) (6-12).

The general consensus of opinion is that the total serum protein level only falls below the normal range when clinical signs of malnutrition are starting to appear. Also, this fall in the total serum protein is mainly confined to children with kwashiorkor. Many severe cases os marasmus have normal total serum protein levels. Probably the main reason the determination has been of such little success is that even through the albumin is depressed the gamma globulin level is often raised due to a coexisting concurrent infection (13). However, the total serum protein is needed if one has determined the albumin-globulin fraction by electrophoresis and wishes to express the albumin or globulin quantitively.

Serum Albumin

A low level of total serum protein is a characteristic of kwashiorkor but is almost entirely caused by a lowering of the albumin fraction (9) (14-17). Needless to say, also the albumin level has been extensively used in nutrition surveys to help evaluate the protein nutrition status. In spite of many nutrition surveys conduced around the world it is still not possible to give a definite answer to the value of the serum albumin as an indicator of early malnutrition. But most investigators believe the determination to be ineffective as an indicator for the detection of early malnutrition (9) (12) (18). However, if only afew albumin values are low in a community there is reason to suspect a deficiency of protein.

Serum Amino Acid Ratio

Investigations of the plasma free amino acids of children with kwashiorkor in several parts of the world showed alterations which were typical for the disease regardless of the differents in the major sourcė of dietary protein (19-24). Changes in the plasma ratio of non-essential to essential amino acids ere observed, mainly caused by a reduction in leucine, isoleucine, and valine, with relatively little changes in the level of nonessential amino acids.

On the basis of these detailed amino acid studies Whitehead & Dean (25) developed a simple paper chromatographic technique in which the ratio of non-essential amino acids to a group of essential amino acids, principally valine, leucine, and isoleucine, were compared. The test has the advantage that it can be performed on only 100 micro-liters of serum. It was noticed that a high ratio was found under conditions of a low protein intake and in severe protein malnutrition. Treatment resulted in a erturn to a normal value. Also, the ratio was well correlated with the percentage weight deficit.

The amino acid ratio was tested in several countries where some investigators found it useful and others did not (26). Inconsistencies with the method were noted in South Africa (27), Lebanon (28), and Turkey (29). It was noticed by all three authors that the type of malnutrition encountered in their areas was more of the marasmic type and therefore gave a normal or near to normal ratio. Thus, another test was found to be only useful under certain conditions and in this case only useful when the diet was low in protein and high in carbohydrate, leading to kwashiorkor. However, these findings testify to the concept that this test enables one to verify whether or not the problem is one of protein deficiency.

Another important point that must be mentioned in relation to the serum amino acid ratio is that a fasting sample of blood is necessary. Amino acid entering the blood after a meal temporarily cancels out an abnormal ratio. The serum sample should be taken at least four hours after a recent meal.

Therefore, there are two important points when one considers the use of the amino acid ratio: a) the test is only useful under conditions where the diet is low in protein and adequate or high in carbohydrate, b) the serum sample used must be obtained from a fasting subject.

Urinary Creatinine-Height Index (CHI)

$$\text{CHI} = \frac{\text{mg creatinine/24 hours excreted by the subject}}{\text{mg creatinine/24 hours excreted by a normal child of the same height}} \times 100$$

The daily excretion of creatinine is closely correlated with total musculature in children and thus could serve as an index of the adequacy of protein intake. Arroyave and Wilson (30) proposed that creatinine coefficient be expressed in terms of height rather than weight since the former is not affected by the amount of adipose tissue. Use of this parameter in three groups of Guatemalan children reveal that an adequately nourished "urban upper-income" group excreted amounts of creatinine that were comparable to those of healthy North American children. Children from a "rural lower-income" group and those with kwashiorkor excreted lower amounts of creatinine per cm of height. Since the authors found a high correlation between 24 hour creatinine excretion and 3 hour excretions calculated to a 24 hour basis, it is conceivable that short term timed collections could be employed in selected population studies.

Viteri, Arroyave, and Béhar (31), and Viteri and Alvarado (32) used the creatinine height index (CHI) with thirty-one children with both kwashiorkor and marasmus. The kwashiorkor children had CHIs from 0.25 to 0.75. Children with clinical marasmus had CHIs which ranged from near normal (0.85) to as low as those with severe kwashiorkor (0.33), indicating a range in degree of protein depletion. Upon protein repletion the CHI approached 1.0 in all children studied. A significant negative correlation between CHI and nitrogen retention was observed during recovery indicating the physiological significance of CHI as an estimate of the degree of protein depletion.

It is obvious that a 24 hour timed urine sample would be almost impossible to collect in the field. But a timed sample of a shorter duration such as a 3 hour sample could possibly be collected. This would first have to be standardized and carefully tested. But since even a three hour timed sample is difficult to collect from small children in the field the author believes this to be the major limitation of the CHI. However, this could be an extremely important test and the problem of a timed urine sample should be carefully studied and tested.

Urinary Hydroxyproline Index (HOP)

$$\text{HOP Index} = \frac{\text{UM hydroxyproline/ml}}{\text{UM creatinine/ml per kg body weight}}$$

The observations that the urinary hydroxyproline peptide excretion is reduced in nutritional dwarfism (33-35) led Whitehead (35) in Uganda to study the problem of hydroxyproline excretion in malnourished children and developed what he called the "index". This should not be confused with the "ratio" wich is simply a ratio of hydroxyproline/creatinine. Whitehead added the parameter of weight to the formula because it had been noted that the hydroxyproline/creatinine ratio fell with age, between 6 months and five years (36-37). Since age is usually not known in many developing countries (Africa mainly) it was important not to have tests dependent upon age. The "index" is contant between 6 months to 5 years.

The "index" when teste din Uganda on both children with kwashiorkor and marasmus showed that it can provide a good index of growth. The index was low in all malnourished children and was statistically related to the deficit of weight.

In a further evaluation of hydroxyproline Howells, Whar ton and McLance (36) tested both the hydroxyproline/creatinine ratio and the hydroxyproline index. They compared the hydroxyproline/creatinine ratio in a 24 hour specimen of urine with a random sample of urine collected during the same period from malnourished children. The ratio in the 24 hour and random samples were closely correlated and it was therefore deducted that random urine samples could be used as valid indicators of the 24 hour hydroxyproline/creatinine ratio. Also a positive correlation was found between the index and the ratio during treatment.

It was also discovered in studies in Uganda that the hydroxyproline/creatinine ratio falls from birth to maturity, also that the index remains relatively constant up to 10 years of age but may be high at puberty before the fall to adult values begins (38): When height was added to the index in the place of weight similar results were obtained (39).

Thus a biochemical test was developed that could possibly distinguish between sub-clinical kwashiorkor and marasmus or between populations where the primary deficiency was either that of protein or calories, i. e. the use of the serum amino acid ratio of the hydroxyproline index, respectively. And when used in a field test in Uganda (39) the two biochemical tests distinguished such a syndrome between two such communities.

However, every biochemical test seems to have its limitations and the excretion of hydroxyproline certainly has its own as well. Further studies in Uganda (40) revealed that when kwashiorkor is complicated by hookworms or malarial infestations a high rather than a low excretion of hydroxyproline was found. Thus a serious limitation of the hydroxyproline index was found. However, studies from around the world should be tried to see whether or not this test has merit as an indicator of the nutritional status.

Urinary Urea Nitrogen/Creatinine Ratio

$$\text{Ratio} = \frac{\text{mg urea nitrogen/ml}}{\text{mg creatinine/ml}}$$

Children subsisting on a low intake of dietary protein have a low excretion of urea nitrogen as determined in a fasting urine sample.

Platt (42-43) measured the urinary excretion of children and lactating women of different nutritional socio-economic conditions and found the ratio of urea nitrogen to total nitrogen to be markedly lower in the groups which had poorer nutrition. Arroyave (44) tested both the urea nitrogen/nitrogen and the urea/creatinine ratios using a single fasting urine specimen from children of both sigh and low socio-economic groups in Guatemala and found the larger difference between the two groups by using the urea nitrogen/creatinine ratio.

Similar results to Arroyave's were found by other workers around the world confirming the usefulness of the urea nitrogen/creatinine ratio to distinguish among different groups of different socio-economic classes (12) (45-48).

Several points should be noted about the urea nitrogen/creatinine ratio. First, the test has the advantage that a single random urine sample can be used. This is easily collected from children. However, the urine sample must be collected from a fasting subject i.e. at least four hours after a meal containing protein. Second, the test is more a measure of dietary intake than an index of the nutritional status. This does not indicate that the test is of no value, but this principle has to be considered . Third, Arroyave (49-50) demonstrated that the excretion of urea is increased by a high urinary flow rate when the protein intake is low but not when it is high. Therefore, the subjects should be instructed to keep their fluid intake to a minimum before collecting urine samples. However, it should be carefully noted that there is still some controversy on this point. This has been reviewed by the author in another publication (51).

Urinary Inorganic Sulphate Sulphur/ Creatinine Ratio

$$\text{Ratio} = \frac{\text{mg inorganic sulphate sulphur/ml}}{\text{mg creatinine/ml}}$$

Fewer references can be found in the literature on the excretion of inorganic sulphate sulphur than on urea nitrogen. However, the principle behind this test is similar to that of urea nitrogen. The inorganic suphate sulphur/creatinine ratio can be determined on a single random urine sample but the sample should be from a fasting subject. This test has the possible advantage over the urea nitrogen/creatinine ratio that it probably reflects the quality of the protein. Especially in relation to the sulphur amino acid in the protein: methionine and cystine.

The National Academy of Sciences —National Research Council (52)— deducted that the inorganic sulphate sulphur/creatinine ratio should be an index of both the quantity and quality of the protein ingested. They concluded that the inorganic sulphate sulphur/creatinine was a better index of utilizable protein than the nitrogen/creatinine ratio.

Miller and Munford (53) and Pellett (54) found a good correlation between the quality of the protein ingested and the excretion of inorganic sulphate sulphur/creatinine ratio. In Pellett's report the following comparisons gave high correlation coefficients: NDp cal % versus inorganic sulphate sulphur/creatinine ratio, sulphur amino acid score versus urinary inorganic sulphate sulphur/ nitrogen ratio, and NDp cal % versus 244 hour inorganic sulphate sulphur excretion.

However, the inorganic sulphate sulphur/creatinine ratio had never been tested under field conditions. If one is dealing with a population whose diet is low in sulphur amino acids the test could be an invaluable tool in helping to assess the nutrition status.

We used (12) (47-48) the test both in children in an elite kindergarten in Nairobi and in the field. The results obtained differentiated among the African, Asian, and European children in the kindergarten and among different groups within Kenya. This was extremely interesting because the diets in Kenya are low in methionine.

Therefore, this biochemical test has a great potential value and certainly deserves further testing around the world under field conditions and in populations with different protein intakes.

General Comments

From the foregoing discussion it can be seen that the possibility of one single biochemical test established the nutritional status is now past history. Therefore, biochemists are now considering a multiple test system, in other words several biochemical tests combined. The trend is towards multiple analysis systems based on automated techniques. This, of course, is not only true of nutritional diagnosis but also of clinical chemistry in general. However, much fundamental research needs to be done before such a system is ready for field studies.

Arroyave (55) in an excellent paper presented at the Nestle Foundation Symposium, Lausanne, Switzerland, outlined such a multiple test system. He suggested using the serum amino acid ratio, hydroxyproline index, the creatinine-height index, and the urea nitrogen/creatinine ratio. However, while being "a step in the right direction" he is limited by the deficiencies of the tests used. These limitations have been outlined above in detail under the description of each biochemical test and should be carefully understood. Therefore, with our present knowledge in biochemistry such a multiple test system would be difficult to propose for field studies (if only used by itself) at least a multiple test system that is effective in all parts of the world.

In the opinion of the author for the present time and probably into the near future the hope of assessing the PCM status in communities lies in the integration of dietary, anthropometric, and biochemical (with a multiple test system) tests. Since biochemical test are more effective if the primary deficiency is one of protein and anthropometry being more effective if the main deficiency is one of calories the combination of the two disciplines could be extremely effective. The result could possible provide such a "profile", "system" or "score", appreciating the combined effect of the various disciplines. Whether or not an international "system" could be developed is a matter of dicussion. This would, of course, be the ideal.

First, two studies should be mentioned where a score has been devised to assess the PCM status. Secondly, two field studies where an attempt was made to assess the PCM status using the various disciplines will be outlined.

Jelliffe and Welbourn (56) outlined a score system for grading PCM where clinical signs and anthropometry were used. The system is advocated to help separate moderate and severe forms of PCM. This score system is limited by the fact that it is only beneficial for distinguishing moderate form severe forms and seems to be of little use in field studies for a more systematic approach.

In a further study McLaren, Pellett and Reed (57) devised a score to differentiate marasmus, marasmic-kwashiorkor, and kwashiorkor. This score is based an clinical signs with biochemical measurements. The system while probably good for local classifications and prognosis would have to be further tested around the world before its use could be advocated. This score is to be used with hospital patients and could certainly not be applied in field studies. However, it should be noted that in some countries the prevalence of PCM is based on hospital records; the statistical value of which is debatable.

In Uganda, Rutishauser and Whitehead (58) chose three areas where the dietary pattern was known: a) an area of protein deficiency, b) an area of calorie deficiency, c) an area where the dietary pattern was changing and becoming like that of a). Complete clinical and anthropometric examinations were done on each child. Biochemistry included the serum albumin, serum amino acid ratio, and the hydroxyproline index. The results were extremely interesting. The serum albumin and the clinical sings were of little help.

However, both the serum amino acid ratio and the hydroxyproline index helped to distinguish between the two communities where either proteins or calories were the major deficiency. Also the anthropometric measurements were helpful. They compared very well with the serum amino acid and the hydroxyproline index.

We (47-48) in Kenya conducted surveys on five locations where complete clinical, dietary anthropometric, and biochemical determinations were done. Again, the clinical signs were of little help. The total serum protein, serum albumin, serum amino acid ratio, hemoglobin, hematocrit, urea nitrogen/ creatinine ratio, inorganic sulphate sulphur/creatinine ratio, and the hydroxyproline index were determined. These biochemical tests were compared to the results from the dietary and anthropometric measurements. Unfortunately, since the ages of all the children were unknown the anthropometric measurement were not as useful as we had hoped. However, an excellent agreement was found between the results from the dietary data when compared to the serum amino acid ratio, and the inorganic sulphate sulphur/creatinine ratio.

The author has attempted to discuss the methods and their limitations for the assessment of the nutritional status confronting the nutritionist today and as can be seen these limitations are numerous. He believes that the multi-discipline approach is the best method to assess the PCM status of communities

In this paper numerical numbers or an actual "profile" have not been given. This is being studied by the author and it is hoped that an actual "profile" can be suggested in a subsequent paper.

APPENDIX I

	Protein	Calories	Constraints
Serum amino acid ratio	Yes	No	Fasted sample
Urinary creatinine-height index CHI	Yes	Yes	3 hr. timed sample
Urinary hydroxyproline index (random sample)	No	Yes	Complicated by hookworm, malaria
Urea nitrogen/creatinine ratio (random sample)	Measure of dietary intake	No	Fasted increased by high urinary flow
Urinary inorganic sulphate sulphur/ creatinine ratio (random sample)	Reflects quality of protein (ref.s.amino acids)	?	Fasted sample

SUMÁRIO

Testes bioquímicos in avaliação do estado de nutrição protéico-calórica.

O autor discute os problemas encontrados quando da avaliação do estado de nutrição protéico-calórica. Os testes bioquímicos discutidos são: proteína total sérica, albumina sérica, relação aminoácido sérico, índice altura-creatinina urinária, índice hidroxiprolina urinária, relação creatinina/nitrogênio uréico urinário, e relação creatinina/sulfato sulfuroso inorgânico da urina.

Chega-se à conclusão de que, no momento e provàvelmente em futuro próximo, esperança de avaliação do estado de desnutrição dos testes dietéticos, antroprométricos e bioquímicos.

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