PROPOSAL FOR A COORDINATED RESEARCH PROGRAMME (CRP) OF THE INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA) ON "STABLE ISOTOPE TRACER TECHNIQUES FOR STUDIES ON PROTEIN-ENERGY INTERACTIONS"

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1. Introduction

This Report provides a rationale and justification for the initiation of a Coordinated Research programme to support studies using stable isotopic tracer techniques to address priority areas of human protein-energy interactions with special emphasis on the problems of human nutrition in developing countries. The Report suggests a modus for establishing such a practically oriented Coordinated Research Programme under the aegis of the International Atomic Energy Agency with concrete suggestions for its organisation and the identification of probable participants in such a programme. The likely sources of additional funding to sustain such an activity viable for a period of 4 to 5 years are also indicated.

2. Objectives of the Report

The main objectives of this Report are to justify the establishment of a practically oriented Coordinated Research Programme (CRP) by the International Atomic Energy Agency (IAEA) which may be supported by the Agency over the period of the next 4 to 5 years. The prime Purpose of such a CRP would be to support studies of human nutrition with particular reference to the problems of nutrition in developing countries in the broad area of protein-energy interactions. Priority problems that are amenable to study will need to be supported in this programme with potential participants in this CRP being drawn from developed and developing countries in such a manner as to promote mutual cooperation and academic interactions in seeking answers or solutions to vexing issues and in the conduct of studies addressing the identified priority problems in human protein-energy interactions.

3. Format of the Report

The Report is organised in the following order. It initially summarises the present state of the art in stable isotope tracer techniques that are applied in studies of human protein-energy interactions, after which it goes on to identify priority problem areas with special reference, to problems of human nutrition in developing countries that are amenable to study and hence deserve support from the IAEA under this new CRP. The Report then attempts to justify the establishment of a practically oriented CRP that needs to be supported by the Agency over a 4 to 5 year period while attempting to identify additional sources of funding for such a CRP. The Report also suggests a mix of potential

participants from developing and developed countries many of them paired together to provide close mutual cooperation and interaction among the paired participants of a developed and developing country. The Report also attempts to identify the technical constraints that a programme such as this may encounter and suggests ways and means to deal with such contingencies.

4. General Overview

Nutritional issues are now becoming an even greater priority in the Third World as well as in developed countries as it comes clear that escalating populations are going to be placing increasing demands on a limited land mass where environmental degradation and salination, erosion or contamination of the most productive areas of the world's food supplies are already evident. The two fundamental issues are the population's needs for energy and protein. Yet much of our current agricultural policies are based on only a crude understanding of the requirements of children and adults because nutritional research has not been a real priority of governments for several decades. Nevertheless, the scientific approach to energy requirements has been revolutionised in the last 10 years and more recently fundamental issues relating to an individual's need for protein have been questioned If one current theory is correct, the adult needs for essential amino acids to maintain health are far higher than expected. If true, this implies (catastrophic) changes in agricultural policy relating to animal production, grain use in animal feed and more limited land for growing crops directly for human consumption.

While these huge dilemmas persist the technology for investigating the problems has now emerged so that it is possible to investigate these issues. The principal needs are for sophisticated approaches to metabolism based on the use of stable isotopes. IAEA is therefore in a unique position to make a major contribution in this field.

5. Stable Isotope Tracer Techniques in Studies of Protein-Energy Interactions.

(i) Protein turnover studies

With the identification of stable isotopes in 1920 their use in biological studies occurred *pari passu* with the development of appropriate instrumentation to estimate quantitatively their relative abundances. Technological innovations in the separation and measurement of stable isotopes led to their use as biological tracers. In 1937, Schoenheimer began tracer experiments in protein and amino acid metabolism and, by 1942, he and his colleagues had demonstrated unequivocally that labelled amino acids when added to the diet resulted in a substantial proportion of the label being retained in the body as an integral component of the tissue proteins. One important and logical consequence of this finding was that proteins exist in a dynamic state in the body. This has resulted in attempts to measure the rate at which proteins turn over within the body. Two principal methods of measuring the rate of protein synthesis using stable isotopic tracers evolved which were based on different biological assumptions. In the so-called constant infusion *'precursor'* method, a labelled amino acid, *e.g.* ^{13}C leucine, is administered and the flux of

that amino acid is estimated from its isotopic abundance in plasma. In the single dose, *"end product"* method the total amino-N flux is calculated from the labelling of urea and ammonia nitrogen in urine after a single dose or infusion of ^{15}N glycine. The underlying biological assumptions and the theoretical problems associated with these methods have been recently discussed both in terms of whole body protein turnover as well as amino acid turnover in man.

Some groups (Millward and others, 1991) are inclined to consider that the ^{13}C leucine method has advantages since the enrichment of its alpha keto acid (viz. alpha keto isocaproate) can be measured in plasma and provide a better estimate of true precursor enrichment for both leucine flux and leucine oxidation. They propose that ^{13}C leucine studies in the whole body permit the investigation of protein and amino acid metabolism with relative confidence and that changes in protein synthesis in the fed state are better estimated using ^{13}C leucine. Thus the coefficient of variations (CV) with ^{13}C is about 7-8% compared with the CV of 36-48% with the end product approach using "N labels. Others (Jackson, 1991) think that the single dose end product method using ^{15}N glycine is an important development with several practical and some theoretical advantages. The latter method is non-invasive, simple to use in practice and can be used for field based studies. The isotopic tracer is given orally and hence follows the natural fate of dietary protein. Since N is used as the tracer the fate of amino acids is followed in terms of the nitrogen pool rather than the carbon pool. However, both methods using stable isotopic tracers of carbon or nitrogen fail to take into account the turnover of proteins whose lifetime is shorter than the period of measurement. This implies that these methods underestimate the true rates of whole body protein turnover. Using these techniques the energy cost of protein turnover has recently been estimated (Waterlow & Millward, 1989). The energy cost of protein synthesis has been estimated directly from stoichiometry of peptide bond formation and indirectly from measurements of the cost of protein deposition in animals. On average there is a five-fold difference between the two estimates, with the indirect estimates being greater than the value of 4 kJ per g for the cost of protein synthesis when regarded as an isolated process and calculated from the stoichiometry. Human studies comparing obese with lean in fasted and fed states, in fasted or fed adult women, in normal subjects vs those with sickle cell disease as well as studies in children who are malnourished compared with rapidly growing children now all provide human data which agree with the indirect estimates of the energy cost of protein synthesis in animals. So the large difference in estimated costs by direct and indirect methods remains an unresolved issue that needs to be tackled.

(ii) 'Essential' and 'Non-essential' amino acids

It has been estimated that the requirements of essential or indispensable amino acids of adults is less than 20% of the total nitrogen intake (Munro, 1985). This means that the requirement of non-essential or dispensable amino acids is over 80% and it is not clear why an adult individual has such a high requirement for non-essential nitrogen. Progressive reduction of protein intake of infants to the point where N balance and growth can no longer be maintained can be reversed by addition of non-essential nitrogen from sources such as glycine or urea to the diet; N from these sources has been shown to be incorporated actively into body protein. In adults, the addition of dispensable amino acids

promoted positive N balance and thus allowed a reduction in energy intake by 10 to 15% in subjects who could not previously maintain N balance on that intake of energy; protein intakes were being maintained at 0.57g of protein/kg/day in these studies (Garza, 1978). These data provide evidence that our traditional concepts of non-essential and essential nitrogen need to be reviewed. The recent studies by the Houston group (Berthold, Hachey, Reeds & Klein, 1990) using uniformly ^{13}C labelled spirulina fed to poultry have elegantly demonstrated how stable isotope tracers can be used to establish the essentiality of certain amino acids in the diet. Their studies suggest that short-term feeding of proteins uniformly labelled with ^{13}C followed by an analysis of rapid turnover over plasma proteins may be used as a probe of *'essentiality'* of individual amino acids in human subjects. More specifically these tracer techniques may help establish the *'conditional essentiality'* of these amino acids during periods of rapid development and growth in infants, during pregnancy and lactation in adults or in relation to the changing levels of energy intake. These techniques may turn out to be the ideal tools to study protein-energy interaction in humans in a wide range of situations encountered in developing and developed countries.

There have been considerable advances in our understanding of the specific metabolic function of individual amino acids most of which have emerged from studies using stable isotopic tracers. These include studies related to the complex metabolic role, of glutamine including its role in the proper functioning of the immune system; the role of arginine in the maintenance of vascular tone, and the functions of the sulphur containing amino acids, cysteine and taurine, as membrane stabilisers and antioxidants. Glycine has an important role associated with growth since growth takes place in a collagen matrix and one third of the amino acid residues in collagen are glycine. During linear growth the demands for glycine are expected to be high. The follow up of labelled plasma lysine and breath CO_2, after administration of oral ^{15}N lysine and intravenous ^{13}C lysine in lactating women, has recently shown that protein intakes of 1.3 g/kg/day are insufficient to support milk protein secretion and to maintain maternal protein metabolism at the same time.

(iii) Urea kinetics and urea salvaging

In normal adults, as protein intake falls, there is a decrease in the rate of urea excretion to maintain nitrogen balance. When the intake of protein in the diet is inadequate or when the metabolic demand for nitrogen is increased in pathophysiological conditions, an increase in the salvaging of urea synthesised by the liver as a potential source of nitrogen has been observed. Increase in urea recycling has also been demonstrated during rapid growth. Studies using ^{15}N urea have demonstrated that effective salvaging of urea-N through the bowel occurs which offsets the impact of reduced N intake. These results indicate a potential limitation in the use of N balance technique for estimating protein requirements and suggest that measurement of urea kinetics using stable isotopic tracers may be a useful and sensitive method for assessing the adequacy of both the quantity and quality of dietary protein intake. The concept of salvaging may have enormous implications to developing countries when N intakes become limited during periods of increased physiological demands.

(iv) Absorption of human milk protein

Stable isotopic tracers have been used to label lactoferrin, an iron binding protein present in human colostrum. Using ^{13}C leucine and $^{15}N_2$ lysine labels it has been demonstrated that *de novo* synthesis of lactoferrin does not occur in the pre-term infant and thus it appears that absorption of intact lactoferrin molecules occurs across the neonatal intestine. Stable isotopic tracers can be used to study the requirements of several other milk proteins, particularly those present in colostrum which may confer immune properties as well as have other important physiological roles in a new-born infant.

(v) Protein metabolism in pathophysiological states

There are several new developments in the use of stable isotopic tracer techniques in clinical situations which have relevance to both developed and developing countries. For example, loss of skeletal muscle is a common phenomenon after surgery or injury. Measurements of muscle protein synthesis using ^{13}C leucine has shown a substantial fall in muscle protein synthesis from the time of initiation of surgery and up to three days post-operative. The post-operative reduction in muscle protein synthesis did not seem to be influenced by the intravenous nutrition during this period. Anaesthetic agents have been shown to inhibit liver protein synthesis. In conditions such as Nephrotic Syndrome enhanced rates of albumin synthesis adequate enough to compensate for renal losses have also been demonstrated using ^{13}C leucine.

(vi) Priority problem areas in protein energy interactions

The question of how much protein and of what quality a child needs to maintain health despite recurrent infections and to grow normally in stature remains a mystery. Epidemiological studies suggest that stunting of height is less likely in children where diets contain animal protein yet metabolic studies of well babies have implied that a vegetarian pattern of food supply is entirely adequate for normal growth. Stunting - affecting 30-60% of Third World children - has been linked to a slowing in mental development also, so the fundamentals of stunting and the role of amino acid intake in promoting skeletal growth and mental development is of huge societal significance.

The problem is not confined to children. If adults have a higher essential amino acid need than we thought, then adults too would benefit from a modest intake of animal protein. Recent analyses suggest that if current perceptions are correct then policies on world food supplies would need to be changed radically with increased emphasis on providing animal protein. There could hardly be a greater issue facing policy-makers, nutritionists and doctors concerned about nutritional issues in the Third World. These issues need to be borne in mind when considering the following proposals.

6. Some Proposals for Future Research

A. Protein metabolism and protein requirements

(i) Amino acid oxidation

Control of amino acid oxidation may be the key to the understanding of many aspects of protein metabolism. The rate of amino acid oxidation and the oxidative drive is determined by the pattern of amino acids entering the pool and also by the energy supply. Increasing intake of energy, more specifically intakes of carbohydrate, reduces N excretion; reducing energy intakes has the opposite effect. The physiological mechanisms of this crucial interaction are not known and need to be elucidated.

(ii) Amino acid requirements

There is general consensus that more work is needed both on the total amount and on the pattern of amino acids required at different ages. It is probable that the pattern of amino acid requirements is different for growth and for maintenance and this needs to be studied. Even with appropriate intakes of amino acids, obligatory N losses are seldom met with an efficiency of more than 70% and the reasons for this are not known. It is also not known whether during pathophysiological stress states it is possible to economise on amino acids by reducing obligatory losses since most studies have been carried out on well-nourished subjects.

(iii) Urea salvaging and recycling

An important area for research is whether urea can be salvaged from the large bowel and made available as N for metabolism. It is important to know more about the extent of urea recycling and salvaging in humans and whether the products can be absorbed and utilised. This is an area ideal for use of stable isotopic tracer techniques.

(iv) Conditionally essential amino acids

It is being increasingly recognised that some amino acids, classically considered as *'dispensable'* or *'non-essential'*, may become limiting under conditions of high demand such as growth or in response to injury. Such *'conditionally essential'* amino acids include glycine, serine, and proline and both collagen as well as acute phase proteins contain disproportionately large amounts of these amino acids. More information is needed on the attributes, requirements and rates of *de novo* synthesis of these amino acids as well as factors that regulate their synthetic rates.

(v) Regulatory influences of specific amino acids

Research is needed to understand the regulatory influence of particular amino acids with known or putative functions (such as glutamine) related to the anabolic drive. Do certain amino acids, such as leucine, have a specific effect on protein synthesis?

B. Growth: weight gain and linear growth

(i) Variability in weight gain

More information is needed on day-to-day variability in weight gain in infants, young children and adolescents, particularly during conditions of enhanced stress, such as infections, trauma, etc. Concomitant variability in protein and energy requirements also needs to be documented.

(ii) Factors limiting protein deposition

Children do not grow faster even when they receive plenty of protein, energy and other nutrients. This is also true of the foetus *in utero* which does not use for growth all the nutrients supplied to it. During conditions of metabolic stress due to severe illness or trauma, it may be impossible to achieve positive nitrogen balance whatever the nutrient supply. Growth is influenced not only by nutrition but also by endocrine secretions. For example, cytokines may also influence this process and explain why infections and trauma retard growth. Weight gain also seems to be influenced by the frequency of feeding. More fundamental research is needed in these areas.

(iii) Requirements for catch-up growth

The amount and composition of tissue deposited during catch-up growth seems to vary. The qualitative and quantitative requirements of specific nutrients in particular amino acid composition of diets that determine the pattern and amount of tissue deposition during catch-up growth need to be elucidated in real-life situations rather than from theoretical calculations.

(iv) Composition and determinants of lean body mass

Recent evidence being incorporated into reports for the International Nutrition Conference suggests that there are many adults, particularly in Asia, who are very thin and can be classified as malnourished. In India about 50% of the population is affected with very high levels of malnutrition also being found in Pakistan Bangladesh and Vietnam. Whether this malnutrition arises from defects in lean body mass accumulation or from effects secondary to a primary deficiency in energy intake is uncertain. The established link to morbidity and mortality, however, makes it of immense importance (Rowett Research Institute, 1991).

It is becoming increasingly evident that further partitioning of lean tissue into muscle and visceral masses is necessary to be able to explain the differing lean tissue composition of individuals with the same body mass index but differing past nutritional experiences. Isotopic tracer techniques need to be devised to obtain estimates of muscle and visceral masses which may help explain the differences in functions, such as BMR, and protein turnover when related to the total lean body mass.

C. Infection

(i) Quantifying losses during infections

The reduced intake, catabolic losses and decreased absorption when children are infected all contribute to the increased requirements for both energy and protein loss of body weight and metabolic balance studies have been the conventional methods used to assess the extent of these losses. Erroneous conclusions are reached when body weight changes alone are used in field situations to assess losses which may be compounded by dehydration or presence of oedema. More information is needed in this area if realistic estimates are to be made of the changes in protein and energy requirements under these conditions.

(iii) Interactions of dietary intakes and cytokine responses

Undernourished subjects show a smaller loss of body N in response to injury. They also have lower levels of pyrexia, leucocytosis and a reduced rise in protein turnover with infections. Some evidence indicates that the cytokine response is impaired. This interaction between energy, protein and amino acid intakes and the cytokine responses clearly need further research.

D. Energy expenditure, physical activity and protein requirements

(i) Energy expenditure of free-living populations

There is no hesitation in stating that we have limited information on the total energy expenditure of free-living individuals, especially in children, adolescents and physically active individuals in developing countries. There are now well-established approaches to integrating information on physical activity but precious little information for predicting when the actual patterns and intensity of activity is under Third World conditions (James & Schofield, 1990). One common misunderstanding, in research terms, is now to assume that the problem can be solved by applying the new doubly labelled water technique on an extensive scale. This ignores scientific and technical problems as well as the issues of the scarcity $^{18}O_2$. The scarce isotope for the doubly labelled water (DLW) method should be channelled to measurements in children and adolescents in view of the world shortage of ^{18}O. There is no simple overall quality control procedure to establish the validity of estimates based upon the DLW method and this method should not be accepted as the *'gold standard'*. This is essential to help reduce the number of injudicious claims made for results obtained with this method.

Support, if any, needs to be provided for studies which use DLW to (a) re-validate the method taking due care to avoid systematic errors resulting from use of FQs rather than RQs and ensuring that the subject is in energy balance; (b) to estimate the extent of sequestration of the isotope in the body, particularly in subjects on high carbohydrate intakes who are in an anabolic state; and (c) to obtain reliable estimates of fractionation of the isotope associated with breath water and/or transcutaneous water losses. This may be a major problem in developing countries in the tropics where body water turnovers are

likely to be high. More small-scale, careful, validation studies are needed before large-scale studies can be justified. Techniques have now been developed involving triply labelled methods which can overcome some of these technical issues.

(ii) Physical activity and protein requirements

Research is needed on the effects of physical activity on the efficiency of energy and N utilisation and on N sparing. Bed rest results in catabolic changes; the interaction between physical activity status and maintenance of lean body mass needs elucidation. There are studies that indicate that physically active children grow faster and that moderately active children spare protein. These observations need to be replicated and confirmed.

7. Organisation of the Coordinated Research Programme

Since a CRP of the Agency is usually developed around a specific scientific topic with between 10 to 20 participants from different countries invited to work together to achieve the aims of this programme, the proposed CRP on protein-energy interactions is also expected to follow the same pattern. Approximately 15 Institutes should be allowed to participate in the proposed CRP, the participants being requested to join in such a manner as to provide equal representation from developed and developing countries. A unique feature of this proposal is that the new CRP has several of the participants *'paired'* or *'twinned'* with a Centre or Institute in a developed country linked with a participating Institute in a developing country. This scheme is expected to provide mutual cooperation and support as well as considerable academic interaction which will result in a substantial transfer of technology and information exchange to developing countries.

The proposed CRP will be initiated following the submission of research proposals in the problem areas identified by the Report. To this end, it is essential that the research priorities in this area and the problem areas identified are circulated to the potential participants. On the selection of the participants, the first CRP meeting should be held to decide on the study programme to be started and the protocols to be decided. The first CRP meeting will provide an opportunity for the investigators to meet, discuss protocols and finalise several details of the studies to be conducted. This will provide an opportunity for close personal discussions among collaborators without having to seek funds for arranging such a meeting between collaborating countries. It is proposed that no more than two paired centres will tackle any one of the priorities identified by this Report. The first CRP with all the selected participants meeting even before the studies have begun will also help provide a greater clarity in terms of which problems are more important than others and which centres are better suited to tackle aspects of the identified problem areas. This may help make the entire CRP a more cohesive and meaningful programme with better chances of success in terms of the whole programme being implemented satisfactorily on a global basis.

Annex 3

8. Additional Sources of Funding

At the fourth IDECG Advisory Group meeting it was suggested that the IDECG could act as a broker between scientists and funding agencies. This suggested role for the IDECG becomes even more significant when the studies to be supported are those considered as research priorities by the scientific groups periodically convened by IDECG. In view of the IDECG's support for the meeting on *'Protein-Energy Interactions'*, this new role of the IDECG needs to be initiated and exploited to provide additional funding for the CRP on *'Protein-Energy Interactions'*. The experience that Nestlé Foundation often works in tandem with the IDECG in identifying its own thrust areas for support, makes the Foundation a possible additional funding agency that may be tapped for this programme.

9. Possible Constraints to the Programme

The technical constraints to this programme are by several orders of magnitude less than that faced by the earlier CRP which was initiated to measure energy expenditure using doubly water. ^{13}C and ^{15}N are relatively inexpensive and are also freely available labelled with no likelihood of a short supply at all. The isotope ratio mass spectrometers dedicated to ^{13}C or ^{15}N measurements can be obtained for less than US $ 100,000 and bench top models are now available which are rugged and hardy and can cope with the vicissitudes of proper and adequate functioning in Third World situations. Support for acquisition of these newer bench top versions by laboratories in developing countries may be initiated as an offshoot of this CRP and will considerably enhance the value of such a programme by an International Agency by appropriate technology transfer. In this context the pairing of participants suggested by this Report and the training programmes supported by the Agency are crucial.

Since the proposed CRP covers the entire gamut of energy-protein interactions, the issues related to the use and availability of DLW cannot but be addressed as they are likely to cause constraints on the successful implementation of this programme. The current short supply of ^{18}O that is acting as a severe constraint on the use of DLW needs to be tackled by requesting the Agency to use its good offices to ensure that production of the isotope is increased. This will also ensure stable and reasonable prices of the isotope. Other international agencies, such as WHO and FAO, need to be contacted as well as advisory groups, such as the IDECG, to create maximum awareness of the shortage of this important tracer so that better production and bulk procurement can then be possible for these studies.

Instrumentation required for the analysis of the biological samples is yet another constraint. The proposed *'twinning'* or *'pairing'* of a developing country scientist with an advanced institute in a developed country may provide a solution to this problem of access to analytical facilities. Quality control may be an important constraint should commercial analytical services be considered for use by the participating scientists.

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