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CO-ORDINATED RESEARCH PROGRAMME ON APPLICATIONS OF STABLE ISOTOPE TRACERS IN HUMAN NUTRITION RESEARCH

Report on the Third Research Co-ordination Meeting

Houston, Texas, USA, 6-9 April 1992

INTERNATIONAL ATOMIC ENERGY AGENCY

Annex 2

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NAHRES-10, IAEA, Vienna (1992)

A report prepared by the IAEA's
Section of Nutritional and Health-Related Environmental Studies
Division of Life Sciences
Department of Research and Isotopes

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SUMMARY

A variety of different applications of stable isotope tracers (particularly ^{2}H, ^{13}C, ^{15}N, and ^{18}O) were presented at the third and final Research Co-ordination Meeting for participants in the Co-ordinated Research Programme (CRP) on Applications of Stable Isotope Tracers in Human Nutrition Research. This CRP is due to phase out by the end of 1992. A final report is planned in the IAEA-TECDOC Series. The present report is therefore confined to a summary of the main points from the discussions during the meeting in question, including suggestions for setting up a new CRP on protein-energy interactions.

A. INTRODUCTION

1. This Co-ordinated Research Programme (CRP) was formally established by the IAEA in October 1988 for a four-year period, i.e. until the end of 1992. Its general objective is to help establish competence in the use of stable isotope techniques, particularly in developing countries, and particularly with reference to applications of ^{2}H, ^{13}C, ^{15}N, and ^{18}O in human nutrition research.

2. Two Research Co-ordination Meetings (RCMs) have already been held, in Vienna (1989) and Bangalore (1991). The third (and final) RCM, which is the subject of the present report, was hosted by the Children's Nutrition Research Center in Houston, Texas, USA. It was attended by all eleven current participants in the CRP (or their representatives). The list of participants and observers is given in Annex 1 and the Agenda in Annex 2.

3. Progress reports (working papers) were presented by all CRP participants who were present at the meeting. In addition, the programme of the meeting included a number of seminars offered by several participants and by staff members of the Children's Nutrition Research Center; a number of laboratory visits were also organized.

4. In contrast to the practice following previous RCMs, it was decided not to publish the working papers in the report of the RCM since a full report on the whole CRP is envisaged shortly (see paragraphs 5-8). For this reason, the present report is confined to a summary of the main points from the discussions, together with some relevant annexes.

B. SUMMARY OF MAIN POINTS FROM THE DISCUSSIONS

FINAL REPORT ON THE EXISTING CRP

5. It was agreed that there will be a final report on the CRP which will be published by the IAEA in the IAEA-TECDOC series. It will comprise a summary report, followed by a series of Final Reports (one for each centre participating in the CRP).

6. The summary report will be drafted by the Scientific Secretary. Input information for this will comprise (i) the individual Final Reports, and (ii) a statement from each Principal Investigator (up to one page in length) summarizing what he/she considers to be the major achievements of his/her research project during the period of the CRP (1988-1992).

7. The deadline for submission of these two contributions to the IAEA is 31 July 1992. They should be submitted in two forms (i) as a hard copy (suitable for direct photo-reproduction, i.e. "camera-ready") and (ii) on a floppy diskette (WordPerfect is preferred, but any other commonly used word-processor would also be acceptable). Prior to this, IAEA will provide detailed guidelines on the length and format required. For those participants who need more time to complete their analyses, there will be an opportunity to substitute pages of their final reports up to November 1992.

8. No other publication on the CRP is foreseen by the IAEA. However, all participants are encouraged to published the results of their own work in appropriate scientific journals.

Acknowledgement of the IAEA's support should be given, e.g. in the following way: This project was supported in part by the International Atomic Energy Agency under Research Contract No. ---- (or Technical Contract No. ----).

DISCUSSIONS RELATING TO THE PROPOSED NEW CRP

General remarks

9. The IAEA is planning to start a new CRP on some aspect of the topic of protein-energy interactions (to be studies with the aid of stable isotopes). At the invitation of the IAEA, a document (annex 3) was drafted by Shetty and James outlining some of the possible priorities and means for organizing the CRP. There was an extensive discussion of this document, which was accepted as providing an excellent framework for the proposed new CRP. The following notes provide further detail.

10. The concept of "twinning" was considered to be an essential component of the CRP. This means that the "backbone" of the CRP will be provided by a group of advanced laboratories in developed countries. Each of these will be twinned with one (or more) participants in a developing country. In general, the developed-country partner will be expected to provide (i) expertise, (ii) analytical services and (iii) training. This assistance will cover ***all*** aspects of the project ranging from design of protocols to the interpretation of the results.

11. Because of this structure, it is expected that participation will be sought, in the first place, among selected institutes in ***developed*** countries, and that these will then be asked to identify a suitable partner (or partners) in a ***developing*** country. Selected scientists in developing countries will also be informed directly about the CRP. In case such a person wishes to apply to join the CRP and is unable to identify a "sponsor" in a developed country, he/she should seek advice from IAEA about this.

12. None of the above detracts from the general objective of the CRP under which it is the ***developing*** country that is intended to be the ***main beneficiary***, i.e. through support of research that is relevant to the needs of that country, as well as through manpower development and technology transfer.

Research priorities and organizational requirements

13. All of the topics mentioned under section 6 of the Shetty/James document (annex 3) are of high priority. However, not all of them are equally amenable to study in developing countries using normally available technology.

14. A general framework for this research is provided by the title: "Impact of the environment on protein-energy interactions: a study using stable isotope tracers". In this context, "environment" is to be interpreted broadly; e.g. it could include such aspects as work load and stress (e.g. due to infection or to some aspect of the physical environment, such as thermal stress). All project proposals should deal with a clearly identifiable problem occurring either globally or locally (in the developing country); there should, of course, also be a clearly defined component that is of nutritional interest.

15. Priority should be given to research topics within the following areas:

15.1. amino acid oxidation
15.2. amino acid and protein requirements
15.3. urea salvaging and recycling
15.4. quantifying protein losses during infections
15.5. energy expenditure of free-living populations
15.6. physical activity and protein requirements

16. Each project proposal should include a draft research protocol with clearly defined research objectives. This protocol should contain sufficient detail to enable the reader to judge whether the objectives can be attained with the available resources and within the time scale foreseen for the CRP.

17. Any appropriate isotope technique may be used in these studies. However, it should be recognized that different techniques give different kinds of information (see annex 4). The choice of technique should therefore be made only after careful consideration of the kind of information expected from the study (or, putting it the other way round, the design of the each project should take account of the kind of information that will be available from the technique being used).

18. Difficulties in the supply of ^{18}O may place some constraints on the use of the DLW method. However, it is hoped to be able to identify participating institutes that already have sufficient stocks of this isotope to be able to sustain them for the duration of the CRP. There are no such difficulties for the other isotopes of interest (deuterium, ^{13}C, ^{15}N).

19. Formal applications to join the CRP should be submitted in parallel (i.e. for both partners in the "twinning" arrangement) and should be accompanied by detailed research protocols covering the work to be done by both partners. The resources available to both partners for carrying out the project (i.e. equipment, manpower, expertise, isotopes and labelled compounds, including assurance of the continuity of supply of all these items for the duration of the CRP) should be specified. It is not necessary for the developing-country participant to have prior experience in the use of stable isotope techniques provided that appropriate experience is available on the part of the developed-country participant.

20. A Research Co-ordination Meeting (RCM) should be organized by IAEA at an early stage of the CRP. Participants should be prepared to present and defend their research protocols at this RCM.

21. Participants in the CRP should be afforded wide discretion in the use of any IAEA financial support that they receive. Legitimate uses include the purchase of isotopes, equipment and supplies, payments to any additional staff that may be needed for the project, and payments (if any) needed in connection with the recruitment of test subjects, and the collection of samples.

22. In contrast to the normal practice in IAEA CRPs, the IAEA is specifically requested to approve the use of its funding for reasonable travel expenses so as to permit both partners to spend some time in each other's institutes (particularly for the developing-country participant to spend time in the other's laboratory for familiarization with the techniques to be used and, at a later stage, to participate in the measurement of the samples and interpretation of the results).

23. IAEA is recommended to explore possibilities for co-operation with other organizations and working groups that have a potential interest in the subject of this CRP, particularly IDECG, WHO and UNU. IAEA should also prepare a list of other potential donors that could be approached on a bilateral basis (e.g. Nestlé, EEC, Wellcome, Thrasher).

24. There will be a need for information exchange in this CRP in the same way as in the previous CRP (mainly relating to bibliographic data from the INIS database). In addition, IAEA is recommended to compile a list of key references relating to this area of research, and to take advantage of Mr. Haggarty's offer to make available his database of literature references on the DLW method.

OTHER ACTIONS IN SUPPORT OF WORK IN THIS AREA

25. IAEA is recommended to organize an expert group to prepare detailed practical guidelines (similar to the IDECG/IAEA report on the DLW method) on the use of stable isotope techniques in study of protein and amino acid metabolism. The names of some possible participants in this expert group were mentioned and noted by the Scientific Secretary.

26. There is a need to improve the certification of some of the IAEA's enriched stable isotope reference materials (RMs), i.e. to obtain agreement on reducing the width of the confidence intervals

surrounding the recommended values. This is particularly needed for the deuterium and ^{18}O labelled water RMs. It was agreed that, in the first place, an effort will be made to reach such agreement within the framework of the new CRP. The first RCM will provide an opportunity for discussion of the technical issues involved.

27. No additional quality control services or RMs are considered necessary at the present time. However, IAEA is encouraged to proceed with the proposal first discussed at the RCM in Bangalore, i.e. to organize an intercomparison based on the use of deuterium-labelled palmitic acid. Mr. Klein offered to make available a set of suitable samples.

28. There will be a continued need for training of scientists from developing countries. Such persons are advised to take advantage of the IAEA's Fellowship Training programme (details available on request).

C. ACKNOWLEDGEMENTS

All participants would like to place on record their sincere appreciation of the excellent arrangements for the meeting and the warm hospitality provided by their local host, Mr. Klein, and his co-workers.

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A G E N D A

Monday, 6 April

9.00 - 9.30 **REGISTRATION & OPENING**

Welcome & Administrative Arrangements

P.D. Klein
R.M. Parr

9.30 - 12.30 **SESSION 1** Chair: P.D. Klein

Adoption of the agenda

Programme overview and status report: R.M. Parr

PROGRESS REPORTS (WORKING PAPERS)

IDECG-related studies using doubly labelled water (DLW)

UK:	P. Haggarty
India:	P.S. Shetty
Malaysia:	Mohd Ismail Noor
Mexico:	M.E. Valencia

14.00 - 17.30 **SESSION 2** Chair: Mohd Ismail Noor

Tour: Body Composition Laboratory (K. Ellis)

PROGRESS REPORTS (WORKING PAPERS) - continuation

Other IDECG-related studies using doubly labelled water (DLW)

Italy:	G. Pastore

Other studies using stable isotope tracers

China:	Zongqin Xia
Germany:	H. Faust

Tuesday, 7 April

9.00 - 12.30 **SESSION 3** Chair: Zongqin Xia

PROGRESS REPORTS (WORKING PAPERS) - continuation

Other studies using stable isotope tracers - continuation

Guatemala:	L. Vasquez Velasquez
Romania:	M. Culea
Uruguay:	P. Moyna
USA:	P.D. Klein

SEMINARS

P.D. Klein:	Overview of CNRC program and research activities
H.K. Berthold	UL ^{13}C algal studies in animals/humans
P.D. Klein	Speed of metabolic adaptation to changes in dietary protein intake
W.W. Wong	Studies of cholesterol synthesis in breast/bottle fed infants
D.L. Hachey	ApoB-100 as a marker for hepatic protein synthesis
S. Brookes	Developments in mass spectrometric instrumentation for studying nutrition with stable isotopes. What is an instrument manufacturer doing to help?

14.00 - ** LABORATORY VISITS followed by an opportunity for individual discussions with the IAEA Scientific Secretary.

- Breath tests, kits, $^{13}CO_2$ gas isotope ratio instruments
- Combustion measurements of carbon/nitrogen isotopes
- Aqueous and organic $^{2}H_2$ and ^{18}O measurements by GIRMS
- Thermal ionization measurements of Ca and Mg isotopes
- Gas chromatography mass spectrometry systems
- Robotic sample preparation

** Open end

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

Wednesday, 8 April

9.00 - 12.30 **SESSION 4** Chair: H. Faust

SEMINARS - continuation

P.D. Klein	Overview of CNRC programme and research activities
W.W. Wong	Studies of cholesterol synthesis in breast and bottle fed infants
H.K. Berthold	Uniformly 13C-labelled algae studies in animals and humans
L.V. Velasquez	Protein & energy metabolism improvement after de-worming
P. Moyna	The possible use of specific labelling techniques in medicinal studies

GENERAL DISCUSSION

Actions required for completing the present CRP
(see appended list of discussion topics)

14.00 - 17.30 **SESSION 5** Chair: P.S. Shetty

SEMINARS - continuation

P.D. Klein	Speed of adaptation to changes in dietary protein intake
D.L. Hachey	ApoB-100 as a marker for hepatic protein synthesis

GENERAL DISCUSSION - continuation

Proposal for a *new* CRP on stable isotope tracer techniques for studies of protein-energy interactions (see appended list of discussion topics)

Evening: Southwestern Buffet - Peter and Roseland Klein

STABLE ISOTOPIC METHODS FOR INVESTIGATING PROTEIN AND

Thursday, 9 April

9.00 - 12.30 **SESSION 6** Chair: M. Valencia

FINAL DISCUSSIONS

REPORT OF THE MEETING

CLOSING OF THE MEETING

14.00 - ** Opportunity for further laboratory visits and discussions with the Scientific Secretary on an individual basis

** Open End

DISCUSSION TOPICS

SESSION 4: DISCUSSION TOPICS RELATING TO THE EXISTING CRP

1. TYPE AND FORMAT OF THE FINAL REPORT
   - do you agree with the idea of publishing an IAEA-TECDOC containing (1) a summary report and (ii) working papers (FINAL reports) from each participant?

2. TIMING OF THE PREPARATIONS FOR PUBLISHING THE FINAL REPORT
   - what deadline shall we set for submission of final reports?

3. ACTIONS NECESSARY BEFORE PREPARATION OF THE FINAL REPORT
   - what actions (*if* any) are necessary by individual participants before they can prepare their final reports?
   - how should the summary report be prepared and what should it say?

4. OTHER PUBLICATIONS PERTAINING TO THE WHOLE CRP
   - is it desirable to prepare any other kind of publication (e.g. a journal article) describing the outcome of this CRP?

5. OTHER PUBLICATIONS PERTAINING TO INDIVIDUAL RESEARCH PROJECTS

6. OTHER MATTERS

SESSION 5: DISCUSSION TOPICS RELATING TO THE PROPOSED NEW CRP

7. "TWINNING" CONCEPT - is this necessary or desirable?

8. SCOPE OF THE CRP - should this be broad or narrow?

9. RESEARCH PRIORITIES - how should these be selected or defined?
   - with respect to the isotope technique(s) to be used
   - with respect to the problem areas to be studied

10. PARTICIPANTS - how may potential participants in this CRP be identified?

11. RESEARCH PROTOCOLS - how & when should they be prepared, and by whom?

12. RESOURCE REQUIREMENTS - what equipment, isotopes, labelled compounds, manpower and expertise are needed:
   - by the partner in the developed country
   - by the partner in the developing country

13. PURPOSE OF IAEA SUPPORT - should this mainly be:
   - for the purchase of isotopes
   - for the purchase of equipment and supplies
   - for TRAVEL of one or both "partners"?

14. CO-OPERATION WITH OTHER ORGANIZATIONS OR WORKING GROUPS
   - are there any other organizations or working groups with which we should try to co-operate?
   - can any of these be approached as potential sources of additional funding?

15. SUPPLIERS OF STABLE ISOTOPES
   - who are currently the most reliable suppliers?
   - what actions (if any) are necessary in order to have a secure supply of isotopes for the duration of the CRP

16. OTHER - what other kinds of action (by IAEA) are needed to support work in this area, e.g.
   - expert meetings
   - quality control services and/or reference materials
   - information exchange
   - training

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"

Prepared at the invitation of IAEA by P. Shetty & W.P.T. James

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 $C0_2$, 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.

(ii) 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.

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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STABLE ISOTOPIC METHODS FOR INVESTIGATING PROTEIN AND AMINO ACID METABOLISM

Some notes prepared by P.J. Reeds at the request of IAEA

1. ^{15}N end product method

Tracers: ^{15}N-glycine or ^{15}N-labelled amino acid mixtures (e.g. protein hydrolysates) given either constantly (Picou and Taylor Roberts) or as a single dose (Fern et al.)

Samples: Urine

Analytical: Total nitrogen, urea and/or ammonia (Stack et al.) ^{15}N isotopic enrichments by gas isotope ratio mass spectrometry.

Derived Values: Total nitrogen turnover, ^{15}N-amino acid balance, whole body "protein turnover".

Advantages: Technically simple, minimally invasive, low isotope costs when glycine is used. Comparisons of urea and ammonia enrichments and oral and intravenous doses may give information with regard to nitrogen enrichment in the visceral and peripheral tissues. Best used to investigate quantitative relationships between nitrogen balance and nitrogen turnover during accelerated growth.

Disadvantages: Relatively non discriminating and measures nitrogen rather than protein. May give results that are biased by the metabolism of the tracer amino acid. With glycine the results are probably biased towards collagen and may also reflect glycine use in non protein pathways of metabolism e.g. creatine, haem, glutathione etc.

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2. ^{13}C amino acid oxidation.

Tracers: Essential amino acids labelled in specific positions with ^{13}C given either orally or intravenously as constant infusion (Motil et al.) or single dose (Irving et al.).

Samples: Breath and urine.

Analytical: $^{13}CO_2$ isotopic enrichments by gas isotope ratio mass spectrometry.

Derived values: Peak enrichment and proportion of dose excreted. The method is essentially the carbon equivalent of nitrogen balance and has been so used in animals and man to investigate amino acid requirements.

Advantages: Simple, relatively low cost and minimally invasive. Very well suited to protocols that demand repeated measurements separated by only a few hours. If 13C-bicarbonate is added to the protocol the method can be adapted to give kinetic information. Comparisons of oral and intravenous doses can give information on the impact of splanchnic metabolism on overall oxidation. With some amino acids (e.g. leucine) measurements of ^{13}C enrichment in the urinary amino acid can be used to derive values for amino acid turnover. Well suited for studies of the impact of non-protein components of the diet on overall protein balance.

Disadvantages: In its simplest form it gives information only on the distribution of the tracer between deposition and oxidation.

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3. Amino acid turnover and protein synthesis

Tracers: Any suitably labelled amino acid. Tracers can be given as a constant infusion or a single dose, intravenously or orally.

Samples: Blood and breath.

Analytical: Free amino acid isotopic enrichments by GC mass spectrometry. Given suitable equipment and laboratory back-up the method can be extended to amino acid metabolites and plasma proteins (e.g- apoB-100, albumin, etc.).

Derived values: Plasma amino acid entry rates, whole body protein degradation and, given a carbon labelled amino acid and breath samples, whole body protein synthesis and amino acid balance. When used with a single dose and frequent blood sampling, especially if a dual oral-intravenous administration is used, extensive kinetic analysis can be made (e.g. Irving et al.). The most appropriate method for specific studies of PROTEIN turnover. Can also be modified to study carbohydrate and/or fat metabolism simultaneously with amino acid metabolism and to study selective aspects of amino acid synthesis.

Advantages: Can be highly discriminatory. Can give information about the synthesis and degradation of circulating proteins, balance between protein synthesis in major protein pools in the body. It can also give information identical to 1 and 2 above. If there is access to biopsies, e.g. of muscle, direct information on tissue protein metabolism can be obtained.

Disadvantages: Invasive and relatively expensive. Can require many blood samples although the size of each sample can be small (e.g. <1 ml.). Requires high quality mass spectrometry and extensive metabolic unit backup. Demands strict diet control during the measurements. However it should be possible to design variants that could be applied out of specific metabolic facilities.

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4. U-^{13}C or U-^{15}N diets

Tracers: Diets in which the organic constituents are uniformly labelled with ^{13}C and the nitrogenous components with ^{15}N.

Samples: Blood, breath, urine, etc.

Analytical: Multiple mass isotopomer analysis of amino acids, amino acid metabolites and proteins. Requires highly sophisticated mass spectrometry facilities.

Derived values: With the exception of oxidation of specific amino acids all the information in 1-3 above. The method allows extensive analysis of amino acid synthesis and interconversion and is the method of choice for studies of essentiality and protocols can be designed that will allow the investigation of amino acid mobilization under stressful circumstances.

Advantages: Yields a substantial quantity of highly detailed information on protein and amino dynamics. Given appropriate analytical facilities it is equally applicable to studies of carbohydrate, lipid and nucleic acid metabolism.

Disadvantages: Very expensive and requires substantial analytical back-up. Many of the restrictions described under 3 above apply to this approach. If used it would need to be highly focused on specific questions.

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