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PROTEIN TURNOVER STUDIES USING STABLE ISOTOPES

The nature of a chemical element is determined by the number of protons (which is fixed) in its nucleus. In contrast, the number of neutrons present in any elemental nucleus may vary. This variability in the neutronic content of any chemical element's nucleus gives rise to isotopes. However, not all known isotopes of an element are stable or naturally present: and presence in nature does not imply stability (*eg* ^{40}K is naturally present but is not stable). Thus, each chemical element consists of two types of isotopes: stable isotopes and radioisotopes. While deuterium is the stable isotope of hydrogen, tritium is a decaying radioactive isotope. In the detection and quantitation of isotopes, the two characteristics of half-life and nature of emissions are employed (Table I)[1].

Table I. Comparison of properties of stable and radioisotopes.

Properties	Stable isotopes	Radioisotopes
Background effect	always present	usually not present
Measurement	usually complex	relatively simple
Multiple labelling	often possible	sometimes possible
Half-life limitation	absent	could be serious
Biohazards	absent	present, sometimes limiting
Dose levels	limiting	not limiting
In vivo monitoring	not possible	sometimes possible
Cost	sometimes high	usually not high

Source: Ref.1

Stable isotopes are thus natural constituents of chemical elements: for example F, Na, P have a single isotope, H, C, N two isotopes and O, S, Fe 3 or more isotopes. An important characteristic of stable isotopes is the extent of their occurrence in nature (natural abundance), expressed in terms of atomic per cent (Table II)[2]. Deviations from the reference isotope abundance are expressed in terms of atom per cent excess which is defined as excess of an isotope over its natural (or reference) abundance. For instance, if the reference value for ^{13}C is 1.084%, then a labelled glycine molecule having 90% ^{13}C in the first carbon would have an excess of 88.9%.

Table II. List of some stable isotopes

Element	Stable isotope	Per cent natural abundance	Element	Stable isotope	Per cent natural abundance
H	1	99.985	S	32	95.0
	2	0.015		33	0.76
				34	4.22
C	12	98.89			
	13	1.11	Fe	54	5.82
				56	91.66
N	14	99.63		57	2.19
	15	0.37		58	0.33
O	16	99.76	Zn	64	48.89
	17	0.037		66	27.81
	18	0.204		67	4.11
				68	18.57
				70	0.62

Source: Ref.2

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Application of stable isotopes in natural science dates back to the immediate years following the discovery of deuterium in the 1930s. The initial studies provided a major breakthrough in the availability of stable isotopic methods for dynamic studies in metabolic process. Stable isotopes are used in biology to measure the elemental composition and also as tracers. In view of the absolute safety, the stable isotopes have many *in vivo* applications (Table III)[1].

Table III. Some applications of stable isotopes.

* Enzyme pathway mapping determination	* Reaction rate
* C_3/C_4 plant photosynthesis (discrimination type indicator)	* Non-protein nitrogen nutrition
* Protein nitrogen metabolism	* Dinitrogen fixation
* Mineral metabolism	* Tracing of pharmaceuticals
* Aminoacid/protein absorption	* Isotope dilution studies
* Nutrition studies in animals and plants	* Breath tests for hydrolysis, oxidation and absorption
* Double labelled water for body composition and energy expenditure	* Body water/milk volume

Source: Ref. 1

Measurement of Stable Isotopes

With the exception of H, C, N and O for which measurement techniques based on physical and atomic properties have been developed, there are two general methods available for analysis of stable isotopes, both based on the differences in their nuclear properties, mass spectrometry, and activation analysis, after neutron bombardment. Both techniques achieve greater development and sophistication aided by computerization. Typical Isotope Radio Mass Spectrometers (IRMS) used for gas analysis and Thermal Ionization Mass Spectrometers (TIMS) for complex matrices which cannot be analysed in IRMS, are the two types of mass spectrometers commonly employed in stable isotope studies.

IRMS is usually dual-inlet dual-collector type capable of extremely high measurement precision. The application of this instrument is in the measurement of isotopically enriched H, C, O, and N introduced into the ion source in gaseous form: H_2, N_2, CO_2. This is a well established technique employed extensively in the study of substrate metabolism.

The *in vivo* techniques of activation analysis require bombarding the target area (hand or other parts of the body) with nuclear particles (primarily neutrons from various sources). The incident neutrons then interact with the stable isotopes of the chemical elements in the body producing, in a very selective manner, radioisotopes which are then measured with appropriate nuclear instrumentation. An example of this application is measurement of body calcium with [illegible]MeV neutron activation analysis. However, measurement of such major body constituents as N, Na, and Ca using *in vivo* techniques require careful assessment of their risk/benefit ratio. It would not be reasonable to expect their use in critical areas involving children, and women of child bearing age[1,2].

Gas chromatography-mass spectrometry (GCMS) is becoming popular for stable isotope tracer studies employing complex matrices which cannot be used with IRMS. When the instrument is operated in the Selective Ion Monitoring (SIM-GCMS) mode, it can provide reasonably precise measurements of isotope ratio in complex matrix. In this method the sample components of interest require conversion to thermally volatile and stable gases and this is usually done by appropriate derivatization.

Uses of Stable Isotopes

Stable isotopes are used in biology as (i) measures of elemental composition; (ii) naturally labelled tracers; and (iii) artificially enriched stable isotope tracers. Stable isotopes have many uses in addition to the [illegible]se in all applications where radioisotopes are used from enzyme pathway mapping to body composition, from reaction rates to turnover rates and from mineral metabolism to nutritional studies in animals (Table III). Accurate measurement of isotopes is an important fundamental requirement for tracer studies. In the case of stable isotopes the measurement of isotope ratios is commonly used.

Stable isotopes are elements with higher atomic number than natural elements and are non-radioactive. Stable isotopes are increasingly replacing radioisotopes in research and diagnosis and they provide more reliable information than radioisotopes with no risks. For certain applications as in pregnancy or in neonates, radioisotopes cannot be used and no radioisotopes are available for elements like oxygen.

Studies on Protein Turnover

Retinol transport proteins

Retinol binding protein (RBP) and transthyretin (TTR) are involved in the transport of retinol (vitamin A) as a protein-protein complex in blood. Both these proteins are synthesized and secreted from the liver, the storage site of retinol[1]. In vitamin A deficiency, apoRBP accumulates in the liver, without any change in hepatic TTR, and it is secreted immediately after vitamin A is reintroduced in the diet[1]. The synthesis and circulating concentrations of RBP and TTR are extremely sensitive to the intakes of energy and protein. This observation, coupled with the rapid turnover of these proteins, makes them potentially good indicators of protein nutritional status[3-5].

Because of the characteristic of their interactions and the lack of appropriate methods to measure the turnover of these proteins, relatively little progress has been made towards a better understanding of the kinetics of the individual proteins and the complex. The information available is based on the use of radioiodinated TTR-bound/free RBP. The results of these studies showed that TTR-bound and free RBP have different replacement times[6,7]. The turnover of RBP is estimated to be 16 μmoles/day which should be equivalent to about 16 μmoles of retinol/day, as against the intake of only 7 μmoles of retinol/day reported in populations from advanced countries. However, the turnover of TTR of 10 μmoles/day is closer to the actual intakes of retinol. When it is believed that retinol in circulation exists as a equimolar complex, different replacement times for different elements of the transport complex point to either a greater catabolic rate of these proteins or increased recycling of retinol. The latter possibility has been suggested by earlier workers[3].

Recently both human and pig plasma samples were found to contain a peptide termed TTR_2, which has a molecular mass similar but not identical to that of TTR. This peptide always occurred bound to RBP and showed fractional synthesis rates (FSR) different from that of TTR and RBP in pigs[8]. This was suggested to be another member of the retinol transport complex or a part of RBP receptor. TTR_2 is the only protein which showed significantly lesser FSR as well as reduced plasma concentration in chronic protein deficiency in pigs[9].

Acute phase proteins

During the metabolic response to injury and infection, there is a marked rise in the circulating concentrations of the hepatically synthesized positive acute phase proteins (APPs) and a decrease in the concentrations of the negative acute phase proteins[10]. The positive APPs constitute a structurally and functionally heterogeneous group and are involved in the removal and repair of damaged tissues, the protection of healthy tissues, and the immune response[11]. Because of the critical role they play during recuperation from injury and infections, these proteins have received a great deal of attention[10-12]. Nearly all studies on positive APPs, however, have been limited to measurements of changes in plasma concentrations in response to injury or infections and during the course of recovery. Hence, although the extent to which the circulating pools of these proteins increase and decrease during injury or infection has been well established, very little information is available on the kinetic changes responsible for these changes. This paucity of information is due to the lack of appropriate methods to measure the turnover of these proteins. The major difficulty in determining the enrichment of proteins in blood is on account of the tedious methods of isolation and purification like salting out, density gradient centrifugation, affinity chromatography and solvent extraction[13]. Also inadequately small amounts of proteins are finally available for analysis with moderate sample size.

Stable isotopes in protein turnover studies

Apart from the radiation hazards, a major concern with exogenous labels is that the addition of the bulky iodine atom alters the physiochemical properties of the protein, thereby altering its susceptibility to proteolysis[14,15]. With the advent of stable isotopes, studies of protein turnover are easily carried out with $^{2}H/^{15}N/^{13}C$-labelled essential aminoacids, particularly in humans[16,17]. Improvements in gas chromatograph-mass spectrometers have allowed such studies to be extended to that of individual proteins[18,19]. The main objectives of studies undertaken by Sivakumar and colleagues [8,9,20] were to (i) use stable isotopically labelled leucine to measure the synthesis rates of TTR and RBP and some acute phase proteins like transferrin (Tr), haptoglobin (Hp) and antitrypsin (At); (ii) develop simple, cost effective methods of purification of plasma proteins using immunoprecipitation followed by SDS-PAGE in young pigs; and (iii) extend the methodology to human infants.

The turnover of proteins has been studied mainly by administering deuterium (a stable isotope)-labelled aminoacid and using the precursor-product model to calculate its rate of synthesis[21]. In this approach, it is necessary to obtain (a) the enrichment of the protein-bound aminoacid tracer at two time points during the linear phase of incorporation of the labelled aminoacid into protein and

(b) the steady-state enrichment of the tracer pool size of the active aminoacid at the site of protein synthesis in the liver. Lack of suitable methods for isolation of adequate amount of the protein of interest from plasma in the pure form to yield sufficient protein-derived aminoacids for mass spectrometric analysis has so far limited such application.

Four or 12 week old pigs were surgically implanted with catheters in the jugular vein and the carotid artery[8,9]. A sterile solution of $^2H_3$-Leucine (Cambridge Isotopes, Woburn, MA, USA) in saline was given as a priming dose of 30 µmoles/kg followed by a constant infusion at 30 µmoles/kg/h for 6-8 h. In young children, a catheter was inserted in peripheral arm vein for blood sampling. The isotope dose was infused with a milk-based feed through a nasogastric tube at a dose as mentioned above for 8 h[20].

The proteins were isolated from plasma by immunoprecipitation and purified by SDS-PAGE. From a single plasma sample At, Tr and Ha were isolated by treating sequentially with antisera against the three proteins[20]. Similarly after removing TTR with complete immunoprecipitation, the remaining plasma sample was treated with RBP antiserum to obtain immunoprecipitate containing RBP and, a new protein called as TTR_2[8]. These proteins were resolved using SDS-PAGE 7% for Tr and Ha and 12% for other proteins. Major bands of each protein having mobility corresponding to the molecular mass of standards were used for the enrichment studies.

Stable isotopic measurements

The isotopic enrichment of apoB-100 achieved a constant value after 2 h of infusion and is parallel to that seen in plasma leucine (Fig.), confirming the previous observations[16]. This provides steady state incorporation of labelled leucine into apoB-100 and reflects the pool size of the active aminoacid. The time course of incorporation of [$^2H_3$] leucine into RBP and TTR (1 and 2) in piglets shows an initial delay after which there was a rapid and near-linear increase in the isotopic content of TTR and RBP (Fig.). These findings justifiy the calculation of FRS based on values over 2-4 h or 3-6 h time points which fall into the linear portion of the curve.

Because of the slower turnover rates of some proteins like albumin or TTR_2, only a modest increase was seen in the tracer/tracee ratio of the protein-bound aminoacid during a 6-8 h isotope infusion. Nevertheless, with the recent improvements in gas chromatograph-mass spectrometers and the exquisite sensitivity achieved with the negative chemical ionization approach, these relatively low tracer/tracee ratios can be measured very accurately[19].

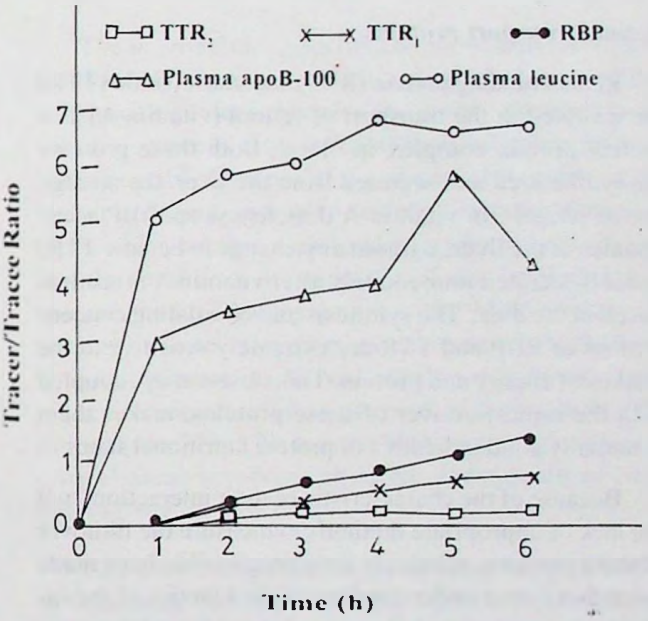


Fig. Time course of typical isotope enrichment into plasma leucine and some proteins

In vivo measurements of the turnover of RBP and TTR (prealbumin) have been previously reported in humans[5-7], cynomolgus monkeys[22] and rats[23]. All these studies used analysis of the decay of the radioiodinated tracer proteins to compute degradation rates. Each study reported substantial difference in the half-lives of TTR and RBP[5]. Depending on the method of iodination, ^{125}I radio labelled proteins showed different affinity[14,15] in binding their receptors and therefore such data were not reliable. The fractional synthesis rates of different proteins are slower when estimated with stable isotope as compared to the earlier data using radioisotopes, confirming the instability of the radiolabelled proteins and emphasising the errors in measurements of turnover obtained using them (Table IV).

The other important observation is that the FSRs of RBP and TTR in younger pigs were much faster than those from older ones. Using radiolabelled tracers, previous studies showed a four-fold difference between the half-life of TTR and RBP in humans or monkeys[5], while that was only two-fold in the stable isotope study[8]. The figures of turnover of TTR and RBP agree more realistically with the estimated 7 µmoles/day intakes of retinol in healthy populations.

The results (Table IV) also show that one of these proteins, At had a very slow turnover rate (10 ± 1.5% pool/day). The FSR was even slower than the rate reported for albumin (which has a very slow turnover)

in a group of young children who had recovered from severe protein energy malnutrition[24]. Ha (50 ± 8%/day) and Tr (19 ± 1.1) had relatively slower FSR than reported earlier[23,24]. These were the first reports in literature on the rates of synthesis of acute phase proteins in young children.

Table IV. Fractional synthesis rates (FSR) of plasma proteins using stable isotopes.

Protein	FSR (% pool/day)			
	Pigs 4 months n=7	Pigs 8 months n=4	Children n=3	Literature values (adult)
RBP	93.4±9.6	56±5	1	139
TTR	47.0±3.1	25±2	–	34
TTR_2	7.0±1.4	6±1.5	–	–
TRANS-FERRIN	–	18±4	19±1.1	24
HAPTO-GLOBIN	–	48±8	50±8	36
ANTI-TRYPSIN	–	–	10±1.5	17
FIBRI-NOGEN	–	–	43±2.0	45*

FSR = (%/h) = [(PE16 - PE13)/(Ep)] x [100/31] where PE16 - PE13 is the increment in the isotopic enrichment of protein-bound leucine over the last 3 h of the infusion and Ep is the plateau enrichment of apoB-100-bound leucine over the same time period.

* By stable isotopic method

Values are Mean ± SE

Source: Refs.8, 9, 20 and 25-27.

Thus the turnover estimates obtained with natural stable isotopes are reliable[8,9,20]. The micro-methodology with sequential immunoprecipitation developed recently will make the GCMS analysis more cost-effective, because plasma samples obtained from a single study could be conveniently used for deriving multiple protein turnover rates. With increasing burden of radioisotopes in the environment and problems of their disposal, applications of stable isotopes is going to be the only way left for the modern world to deal with the future needs of tracer studies to solve health problems.

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This write-up has been contributed by Dr. B. Sivakumar, Deputy Director (Senior Grade), National Institute of Nutrition, Hyderabad.

ABSTRACTS

Some Research Projects Completed Recently

Expression of antimetastatic marker-nm 23 and prolactin in human breast carcinoma : Its relation with relapse, survival and other prognostic factors.

The study was carried out in 100 patients (25 with benign breast disease and 75 with breast cancer) to investigate the combined utility of an antimetastatic marker nm23 and prolactin – a marker of tumour aggressiveness, in the management of patients with benign breast disease, early and advanced breast cancer. The parameters studied included prolactin (PRL) estimation – pretherapeutic and serial monitoring as an indicator of disease status, immunohistological localization of PRL, expression of PRL mRNA and nm23 mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR).

Hyperprolactinaemia was observed in 52% (13/25) of patients with benign breast disease and 55% (41/75) of breast cancer patients. Hyperprolactinaemia was observed more in patients with advanced stage cancer (63%) compared to those with early stage disease (41%). The levels of PRL increased with appearance of recurrence/metastatic disease and decreased in patients who responded to therapy. It was found that rise in PRL level preceded disease progression. The PRL level remained elevated throughout the course of the disease in patients who did not respond to adjuvant therapy.

In 72% (18/25) of patients with benign breast disease and 79% (53/67) of breast cancer patients the tumours showed immunoreactivity with PRL antibody. A total of 56% (40/71) breast cancer tumours and 40% (4/10) of the benign tumours expressed PRL-mRNA. Immunoreactivity of nm23 was found to be 84, 69 and 80% in benign, stage II and advanced stage cancer patients, respectively. The nm23 mRNA expression was observed in 70% of benign and 100% breast cancer patients. Eighty six per cent (44/51) of the nm23 positive and 56% (9/16) of the nm23 negative tumours showed immunoreactivity with PRL antibody.

The results showed that PRL is a superior marker for monitoring the disease course whereas nm23 cannot be used as an antimetastatic marker but is a marker of tumour aggressiveness.

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Publications:

1. Bhatavdekar, J.M., Patel, D.D., Vora, H.H., Shah, N.G., Karelia, N.H., Ghosh, N., Suthar, T.P., Dave, R.S., Mehta, R.H., Chikhlikar, P.R. and Balar, D.B. Comparison of prognostic value of plasma prolactin with that of receptors for prolactin, epidermal growth factor, estrogen and progresterone in patients with stage III breast cancer. *Breast Dis 8*: 317, 1995.

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Targeting of glycosylated liposomes encapsulating metal chelating drugs to the site of toxic metal storage in the biological systems.

The study was carried out in male albino mice to achieve improved therapeutic and safety profile of metal chelating drug – meso-2,3,dimercaptosuccinic acid (DMSA) in the management of cadmium toxicity by using glycosylated liposomes as carriers for their targetted delivery.

Glycosylated liposomes encapsulating DMSA were prepared involving coupling of glycosides to liposomes and their safety was evaluated in terms of blood and biochemical parameters and trace metal levels in the body, their stability and *in vitro* interaction of liposomes with isolated hepatocytes.

Cadmium toxicity was produced in the mice by injecting cadmium chloride (0.01 mmole/kg) intraperitoneally. The chelating drug DMSA was given as (i) DMSA encapsulated in glycosylated liposomes, (ii) DMSA via plain liposomes; and (iii) plain DMSA.

The glycosylated liposomes encapsulating DMSA could be successfully targetted to the intracellular site of cadmium accumulation *ie* hepatocytes (because of the presence of free terminal sugar – galactose for which receptors exist on the hepatocyte cell surface). This resulted in the enhanced mobilization of cadmium from the body organs (lever, kidneys and spleen) and from hepatocytes. It also produced enhanced excretion of cadmium through urinary and fecal route. The efficacy of DMSA encapsulated in targettable liposomes was found to be better compared to that given via plain liposomes or in the form of free material. The DMSA given as free material and via plain liposomes resulted in lowering of biochemical (protein, glutathione, lipid peroxide levels) and haematological (haemoglobin level) parameters in cadmium exposed mice, whereas the DMSA given through glycosylated liposomes was able to maintain the levels of these parameters to their normal values.

The DMSA given via liposomes in both the forms (plain liposomes and glycosylated liposomes) lowered the levels of copper and zinc enhanced by cadmium treatment, thereby preventing the disturbances in essential trace metal imbalances. Also these liposomes when administered empty to normal animals did not show any toxicity to disqualify their use as carrier for metal detoxification.

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Publication:

Behari, J.R., Srivastava, S., Gupta, S., Misra, M. and Srivastava, R.C. Influence of 2,3-dimercaptosuccinic acid esters administered via liposomes on biochemical alterations and cadmium levels in cadmium pre-exposed rats. *J Sci Ind Res 3(1)*: 27, 1995.

ICMR NEWS

The following meetings of various technical groups/ committees of the Council were held:

Meetings of the Task Forces (TFs)/Project Review Committee (PRC)/Expert Group (EG)/Scientific Advisory Committee (SAC)/Scientific Advisory Group (SAG) held at New Delhi:

TF on Suicide	February 16, 1999
PRC on Mental Health, Environmental Health, Neurology and Biomedical Engineering	February 17, 1999
PRC on Haematology, Human Genetics and Physiology	February 18, 1999
TF on Drug Trials in Malaria	February 22, 1999
TF on District Nutrition Project (Phase I)	February 22-23, 1999
EG on Estimates of Disease Burden due to Shigellosis in a Rural Community in the Andamans	March 4, 1999
SAC of the ICMR-NIC Centre for Biomedical Information, New Delhi	March 9, 1999
SAG on Pharmacology and Toxicology including Traditional Medicine	March 12, 1999

Participation of ICMR Scientists in Scientific Events:

Dr. P.R. Gangakhedkar, Sr. Research Officer, National AIDS Research Institute (NARI), Pune, participated in the workshop on Communication and Public Information Issues on HIV Vaccine Trials at Geneva (March 1-3, 1999).

Dr. N.K. Ganguly, Director-General, ICMR, participated in the 21st meeting of the Scientific and Technical Advisory Committee of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases at Geneva (March 1-4, 1999).

Dr. D.A. Gadkari, Director and Dr. B. Lalitha Rao, Dy. Director, National Institute of Virology, Pune and Dr. Pradeep Das, Asstt. Director, National Institute of Cholera and Enteric Diseases, Calcutta, participated in the International Conference on Emerging Infectious Diseases in the Pacific Rim at Bangkok (March 2-4, 1999).

Dr. V. Ramesh Bhat, Dy. Director (Sr.Grade), National Institute of Nutrition (NIN), Hyderabad, participated in the 3rd joint FAO/WHO/UNEP International Conference on Mycotoxins at Tunis (March 3-6, 1999).

Dr. P. Bhaskaram, Dy. Director (Sr. Grade) and Dr. K.V.R. Sarma, Asstt. Director, NIN, Hyderabad, participated in the XIX International Vitamin A Consultative Group meeting at Durban (March 8-11, 1999).

Dr. V. Kumaraswamy, Dy. Director, Tuberculosis Research Centre, Chennai, participated in the meeting of the WHO Task Force on Community Directed Treatment of Lymphatic Filariasis and Onchocerciasis at Geneva (March 8-11, 1999).

Dr. A.R. Risbud, Asstt. Director, NARI, Pune, participated in the International Conference on HIV Vaccine Development : Global, Regional and Thailand Update 1999 at Bangkok (March 15-19, 1999).

Dr. Lalit Kant, Sr. Dy. Director-General, ICMR Hqs., New Delhi, participated in the conference on Vaccine Development and Delivery : Partnership for the 21st Century at Bellagio (March 15-18, 1999).

Workshop/Seminar:

Fourth Indo-German Workshop on "Oncology into the Next Millennium" was organised at Lonavala during February 21-25, 1999.

A joint ICMR/ICSSR National Seminar on "Status of Health Care in India" was organised at New Delhi during March 9-10, 1999.

ICMR AIDED SYMPOSIA/SEMINARS/WORKSHOPS/COURSES/CONFERENCES

Symposium/Seminar/Workshop/ Course/Conference	Date & Place	Contact Address
Indo-French Symposium on Multiple Drug Resistance and Emerging Diseases	February 28-March 4, 1999: (at New Delhi)	Dr. S.K. Sahani, Executive Secretary, Indian National Science Academy, New Delhi-110002.
Technical Training Workshop on Industrial Hygiene Measurements and National Seminar on Occupational Safety and Health	March 1-6, 1999: (at Chennai)	Dr. Kalpana Balakrishnan, Organising Secretary of the Workshop, Sri Ramachandra Medical College and Research Institute, Chennai-600016.
International Symposium on Prudent Use of Antibiotics	March 5-7, 1999: (at Jaipur)	Dr. Kunal Kothari, Organising Secretary of the Symposium, Department of Medicine, S.M.S. Medical College, Jaipur-302004.
VI Chandigarh Symposium on New Biology	March 19-20, 1999: (at Chandigarh)	Prof. R.C. Sobti, Organising Secretary of the Symposium, Department of Biotechnology, Panjab University, Chandigarh-160014.
Workshop on HIV/AIDS/STDs and Healthy Life Style	April 10-11, 1999: (at Berhampur)	Dr. R.M. Tripathy, Organising Secretary of the Workshop, Department of Social and Preventive Medicine, MKCG Medical College, Berhampur-760004.

COUNCIL'S TRAINING PROGRAMMES FOR 1999-2000

Leprosy

At the Central JALMA Institute for Leprosy, Agra:

- Training Course for Medical Officers Working under the National Leprosy Eradication Programme (April and September, 1999).

Reproductive Biology

At the Institute for Research in Reproduction, Mumbai:

- Training Course in Molecular Techniques in Ovarian Function (September 27-October 2, 1999).
- Training Course on Cytological Detection of Reproductive Tract Infections (December 13-17, 1999).

Endocrinology

At the National Institute of Nutrition., Hyderabad:

- Annual Certificate Course on Endocrinological Techniques and their Application (August 1 - September 15, 1999).

Nutrition

At the National Institute of Nutrition, Hyderabad:

- M.Sc. in Applied Nutrition (June 1, 1999-February 28, 2000).
- Postgraduate Certificate Course in Nutrition (December 1, 1999-February 28, 2000).

Laboratory Animal Technology

At the National Centre for Laboratory Animal Science, National Institute of Nutrition, Hyderabad:

- Training Course for Laboratory Animal Technicians (June 14-July 30, 1999).
- Training Course for Laboratory Animal Supervisors (September 1-November 30, 1999).

Medical Entomology:

At the Vector Control Research Centre, Pondicherry:

- Postgraduate Diploma Course in Medical Entomology (from July 1999).

Biomedical Statistics

At the Institute for Research in Medical Statistics, Chennai:

- Basic Course in Statistics for Medical Officers (July 1999).
- Training Course in Field Epidemiology (November, 1999).

Haematology

At the Institute of Immunohaematology, Mumbai:

- Training Course in Transfusion Medicine for Blood Bank Medical Officers (August 31-October 29, 1999).
- Training Course in Blood Group Serology and Blood Bank Methodology for Blood Bank Technicians (August 31-September 30, 1999).
- Training Course in Advanced Haematology and Immunohaematology (October 11-29, 1999).

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	Price(Rs.)
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Growth and Physical Development of Indian Infants and Children (1972, Reprinted 1989)	10.00
Studies on Weaning and Supplementary Foods (1974, Reprinted 1996)	15.00
A Manual of Nutrition (Second Edition 1974, Reprinted 1995)	6.00
Low Cost Nutritious Supplements (Second Edition 1975, Reprinted 1996)	5.00
Menus for Low-Cost Balanced Diets and School-Lunch Programmes Suitable for North India (Second Edition 1977, Reprinted 1994)	4.50
Menus for Low-Cost Balanced Diets and School-Lunch Programmes Suitable for South India (Fourth Edition 1996)	6.00

Some Common Indian Recipes and their Nutritive Value (Fourth Edition 1977. Reprinted 1995) by Swaran Pasricha and L.M. Rebello	10.00
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