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APPLICATION OF MOLECULAR BIOLOGICAL TECHNIQUES IN THE STUDIES ON VECTOR MOSQUITOES – CURRENT STATUS

Correct identification of vectors of malaria, filaria and viral diseases is very essential to determine the vectorial significance of different species particularly in complexes. Recognition of resistant strains of vector mosquitoes to insecticides and more rational and selective use of insecticides are the methods of optimising vector control programmes most of which are heavily dependent on insecticides. Similarly, the correct identification of parasite species in vector mosquitoes is necessary to understand the dynamics of transmission and to device appropriate intervention measures. In general, the sporozoites of different malarial parasites of man and animals in vector mosquitoes cannot be differentiated morphologically. In the case of filarial parasites, it is possible to distinguish them up to the generic level on the basis of the shape, size and caudal morphology of the infective larvae. But, developing and infective stage larvae of different species in the same genus are often morphologically identical and hence difficult to differentiate. With the advent of biotechnology, molecular biological techniques have emerged as potential tools to address some of these problems. The present review pertains to the application(s) of molecular tools in the study of vector mosquitoes and the parasites transmitted by them.

Discrimination of Species Complex

The various techniques used for discrimination of mosquito species complex are shown in Table I. There are ten cryptic species *viz.*, *Anopheles farauti* no. 1-7, *An. punctulatus*, *Anopheles* sp. near *punctulatus*, and *An. koliensis* among the members of the group, *Anopheles punctulatus*, the most important vector of malaria in the southwest Pacific. Studies on allozyme patterns confirmed this and revealed another 4 more isomorphic species[1-3]. DNA probes, constructed for all 10 species of this group have proven to be useful for large scale identification of field collected specimens[4-6]. Another technique based upon PCR-restriction fragment length polymorphism (PCR-RFLP) has been found sensitive enough to differentiate species in complexes. A PCR-RFLP can be generated from as little part of the mosquito as a single leg[7].

Table I. Discrimination of mosquito species complex

Species complex	Technique	Sensitivity/ Specificity	Source
An. punctulatus	Allozyme	High	1,2,3
-do-	DNA probe	High	4,5,6
-do-	PCR-RFLP	Significant	7
An. gambiae	PCR	Significant	8
An. dirus	DNA probe	1-5 ng of DNA	9
An. sinensis	DNA probe	7.5 ng of DNA	10
Ae. aegypti	RAPD-PCR	Accurate	11,12

PCR-amplification for identification of species of *Anopheles gambiae* complex (*An. arabiensis*, *An. merus*,

An. gambiae and *An. quadriannulatus*) has been found to be sensitive, specific and easy to perform[8]. A Simple chemiluminescent detection system was developed for identification of *Anopheles dirus* complex and identified species B, C and D in Thailand, using horse radish peroxidase-labelled DNA probes[9]. These probes detected 1-5 ng of target DNA, a sensitivity level that was comparable with the radioisotopic detection system. This method allowed identification using third or fourth instar larvae as well as the adult head, thorax or abdomen. Another DNA probe was developed for discriminating the two sibling species of *Anopheles sinensis* viz., *An. sinensis* and *An. anthropophagus*. This probe hybridized with all stages of *An. sinensis* DNA, but with *An. anthropophagus* DNA it had a very weak hybridization signal[10]. This probe was very sensitive and could detect as little as 7.5 ng of *An. sinensis* DNA, which represents only 0.67% of the total DNA from a single mosquito.

A method using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) markers, was developed to estimate the number of sibling families of *Aedes aegypti*, a species that distributes its eggs of a single batch from a given gonotrophic cycle among several locations[11,12]. By applying this technique it was possible to estimate the number of full-sibling families contained in oviposition traps. It accurately estimated the number of families when family sizes were small and variable.

Detection of Insecticide Resistant/Susceptible Strains

The enzyme markers of insecticidal resistance/susceptibility are shown in Table II. The enzyme glutathione-S-transferase (GST) is responsible for conversion of DDT to DDE in both the susceptible and resistant strains of *An. gambiae*[13,14]. There are seven GSTs and all of them were found to possess different degrees of DDTase activity. An 8-fold increase in total DDTase activity has been reported in the resistant strain compared to the susceptible one. The data show that both DDT resistance and the increased levels of GST activity are stage specific, suggesting that different GSTs may be involved in DDT resistance in adults and larvae. It was concluded that DDT resistance is conferred by qualitatively different glutathione-S-transferases which are present in higher concentration.

Table II. Detection of insecticide resistant/susceptible strains of vector mosquitoes

Vector species	Marker enzyme	Source
An. gambiae	Glutathione-S-transferase	13,14
Culex sp.	Esterases A2 and B2	15
Cx. quinquefasciatus	-do-	16
Cx. pipiens	Esterase B1	17
Cx. tarsalis	Malathion carboxylesterase	18,19
Cx. quinquefasciatus	Beta-esterase	20

A study with five resistant strains of *Culex* species to DDT and one insecticide susceptible strain, has shown that esterases A2 and B2 interact with a range of insecticides to a similar extent, hence both have a role in resistance[15]. The B2 esterases are more similar between strains, but some of these are also kinetically distinct. Data on the purified esterases from a range of strains suggests that A2 and B2 are present in a single strain. Further, identical restriction fragment length patterns have been observed in the amplicon containing the B2 esterase from *Culex* strains worldwide. Esterases A2 and B2 have been purified from three resistant strains of *Cx. quinquefasciatus* and characterized[16]. Substrate kinetic studies as well as the kinetics of insecticide interaction demonstrated that the esterases A2 and B2 differ both from each other and in the forms found in the three resistant strains.

In order to determine whether the esterase B1 in *Cx. pipiens* mosquitoes from different countries is over-produced due to amplification of the same DNA haplotype, the amplified region encompassing the structural esterase B1 gene was characterized by restriction mapping and RFLP[17]. The results showed that the same amplified haplotype is present in mosquitoes with an esterase B1 protein, independent of their geographical origin (French Guiana, Venezuela, Puerto Rico, California and China). It was concluded that B1 amplification has a unique origin, either in America or in Asia, and has subsequently spread by migration.

Malathion resistance in a strain of *Cx. tarsalis* is primarily due to the activity of a malathion carboxylesterase (MCE)[18]. The resistant strain was 150 times more resistant to malathion than the susceptible strain. Chromatofocusing indicated that the resistant strain has 2 MCEs, while the susceptible strain possesses only one MCE. The MCE, unique to the resistant strain, hydrolysed malathion 18 times faster than MCE common to both strains, suggesting that malathion resistance in *Cx. tarsalis* is due to the presence of a qualitatively different esterase in the resistant strain. Further study on this enzyme[19] has indicated that it does not hydrolyse alpha-naphthyl acetate, a substrate commonly used to detect increased levels of esterases in other organophosphorus resistant insects.

Studies carried out to understand the involvement of beta-esterase-mediated hydrolysis as a mechanism of pyrethroid resistance in *Ae. aegypti, Cx. quinquefasciatus* and *An. stephensi* (in individual larvae and adult females and males) indicated that ester hydrolysis of permethrin and deltamethrin is a predominant mechanism of pyrethroid resistance in the larvae of *Cx. quinquefasciatus* but not in *Ae. aegypti*[20]. However, a marginal role for esterases is indicated in the larvae of *An. stephensi*. A PCR assay performed between the allele-specific primers and flanking primers known to be nested within exon 7 of the GABA receptor/chloride ion channel gene Rdl which contains the cyclodiene resistance associated mutation in *Ae. aegypti* has been observed to amplify the specific allele and allow the detection of the single base pair mutation[21].

Detection of Parasites in Vector Mosquitoes

Viruses

The various methods used for detection of viruses in vector mosquitoes are shown in Table III. A colorimetric dot-assay, developed following PCR method, has been found to detect 0.1 pg of Eastern equine encephalomyelitis virus (EEEV) RNA[22]. This method is 250-fold sensitive than electrophoretic procedures. While RNA of Venezuelan equine encephalitis, St. Louis encephalitis, Keystone, Flanders, Tensaw and Western equine encephalitis viruses were not detected by this assay, the RNA of EEEV (Ten Broeck) was detected at the 10-ng level. The detection of EEEV in the liver of an infected emu *(Dromaius novaehollandiae)* and in field-collected *Culiseta melanura, Cx. erraticus* and *Cx. nigripalpus* that were analysed concurrently as bind samples by tissue culture plaque assay and by PCR dot analysis proved that the assay is sensitive and can be used to detect infected mosquitoes. The assay can detect even a single infected mosquito in a pool of 1000 uninfected mosquitoes.

Table III. Detection of viruses in vector mosquitoes

Virus & host	Technique	Sensitivity/ Specificity	Source
EEEV Mosquito	PCR	0.1 pg RNA of EEEV only or 1 infected mosquito/1000	22
LACV mosquito	RT-PCR	Detects 1 day postin-oculation	23
-do-	EIA	Detects 2 days postin-oculation	23
DEN Mosquito	ELISA	1/1000 dilution of hom-ogenate, but cross reacts with all 4 DEN & JE viruses	24
DEN & JE Mosquito	ELISA	Readily screens antigens in single & pooled speci-mens	25

EEEV: Eastern equine encephalomyelitis virus;
LACV: LaCrosse virus; DEN: Dengue; JE: Japanese encephalitis

A reverse transcription-polymerase chain reaction (RT-PCR) assay has been developed and compared with enzyme immunoassay (EIA) and virus isolation methods for detecting LaCrosse virus (LAC) in mosquito pools[23]. All the three techniques were able to detect a single LAC-infected *Aedes triseriatus* mosquito in a pool of 99 negative mosquitoes. The LAC RNA was detected 1 day post-infection by RT-PCR, whereas EIA detected LAC antigen 2 days post-infection. Further, the RT-PCR and EIA were able to detect LAC RNA and protein, respectively, from mosquito samples which were subjected to 7 freeze-thaw cycles, and RT-PCR was able to detect LAC RNA from mosquito samples which remained at room temperature for up to 7 days.

A rapid ELISA could detect dengue virus in a 1/1000 dilution of mosquito homogenate[24]. However, cross reaction among all 4 dengue serotypes and Japanese encephalitis virus was also noted. An indirect ELISA for detecting dengue (DEN) virus in *Ae. aegypti* and *Ae. albopictus* and Japanese encephalitis (JE) virus in *Cx. tritaeniorhynchus* (experimentally infected) has been developed[25]. DEN antigen from 4 strains of dengue virus and JE antigen were captured with a polyclonal anti-flavivirus IgG and detected with a monoclonal antibody (4G2) that reacts with all flaviviruses. The results indicated that the antigen-capture ELISA could readily screen for DEN antigen in individual and pooled mosquitoes.

Malaria parasites

Plasmodium infected mosquitoes were detected by using a ^{32}P-labelled cloned DNA probe (Table IV). This technique was shown to detect infection even when one out of 25 mosquitoes had the parasites[26]. The head and thorax portions of dried *An. gambiae* mosquitoes, infected with *P. falciparum*, were processed by PCR and the infection was detected by autoradiography[27]. The detection level was, ten sporozoites per mosquito and the head and thorax portions of mosquitoes that contained matured *P. falciparum* oocysts (without sporozoites) did not give positive signal, indicating that the test detects only infective mosquitoes. In an another study, application of dot-blot hybridization with cloned genomic DNA labelled with photobiotin could detect 2 pg

of sporozoite DNA of *P. falciparum* in dried *An. dirus* mosquitoes or 1 infected mosquito in a pool of 25 without DNA extraction[28]. It has been concluded that by using this method, a single mosquito, squashed directly on a nitrocellulose filter, is enough for the detection of sporozoites while the mosquitoes triturated in reduction buffer are sufficient for the screening in a large number of samples. By means of enzymatic amplification of *P. falciparum* DNA (using the PCR), it has been possible to detect a minimum of 10 sporozoites or a single oocyst dissected from a *P. falciparum*-infected *An. dirus*.

Table IV. Detection of malaria parasites in vector mosquitoes

Parasite stage & host	Technique	Sensitivity/ Specificity	Source
Plasmodium Mosquito	^{32}p-labelled DNA	1 mosquito/25	26
P. falciparum Sporozoites *An. gambiae*	PCR	10 sporozoites/ mosquito; did not detect oocysts	27
P. falciparum Sporozoites *An. dirus*	DNA probe	2 pg of sporozoite DNA or 1 infected mosquito/25	28
P. vivax CS antigen *An. stephensi*	IRMA	Sporozoites in mature oocysts a day prior to liberation or in salivary glands or after dissection of salivary glands	29
P. inui Sporozoites *An. dirus*	ELISA	50 sporozoites or mosquito/50	30

Nanda *et al*[29]., carried out a two-site immunoradiometric assay (IRMA) with *An. stephensi* fed on *P. vivax* blood to ascertain at what time in the sporogonic cycle, circumsporozoite (CS) antigen can be detected and to detect CS antigen in mosquitoes squashed on filter paper (FP) and cellulose acetate membrane (CAM). The CS antigen was detectable only in mature oocysts (a day prior to sporozoite liberation) and in salivary gland sporozoites. Dissected sporozoites, adsorbed on FP/CAM, could also be detected by this method.

One and two site ELISAs have been developed for screening mosquitoes for infection with malarial parasite using monoclonal antibodies (MAbs) produced against sporozoite of *P. inui* (N34 strain) infecting *An. dirus*[30]. A one-site indirect labelled avidin-biotin (LAB) assay detected the infected mosquitoes at a sensitivity of less than 50 sporozoites. A comparable level of sensitivity was obtained using an excessive amount of enzyme conjugated MAb in a one-site direct assay. A two-site LAB assay using a crude preparation of biotin labelled MAb and a commercially obtained enzyme-labelled avidin detected about 180 sporozoites, whereas a two-site assay based on a custom-labelled enzyme-MAb conjugate detected about 90 sporozoites. The reliable detection level for pooled mosquito assays was one infected in 50 specimens for the one-site assays and one in 100 for the two-site assays. The indirect LAB ELISA was effective in discriminating gut restricted oocyst infections from the disseminated sporozoite infection in mosquitoes.

Filaria parasites

A PCR-based detection system using *Ssp* I repeat DNA sequence of *Wuchereria bancrofti* has been described for the detection of 1to 6 third stage larvae (L3) of *W. bancrofti* added to 50 mosquito heads (*Ae. polynesiensis*)[31] (Table V). The results indicated that this assay can be a semi-quantitative one for detecting the number of third stage larvae present in pools of mosquito vectors. No cross hybridization or PCR amplification with filarial DNA from any other genus was reported.

Table V. Detection of filarial parasites in vector mosquitoes

Parasite stage & host	Technique	Sensitivity/ Specificity	Source
W. bancrofti L3 larva in *Ae. polynesiensis* & mF in blood	*Ssp* I PCR	Detects 500 pg of DNA in blood and one L3 in pools of 50 uninfected mosquitoes	31
Dirofilaria sp. & *D. immitis* L3 larva in mosquito & mF in blood	PCR	Single infected mosquitoes stored desiccated at RT for 7 months; did not detect *D. temuis*	32
D. repens mF in blood & L3 larva in Mosquito	DNA hybridization	Detects single mF in blood or single L3 in mosquito	33
B. malayi	*Hha* I PCR	When radioactively labelled-species specific & sensitive detects as little as 200 pg in a pool of 50-100 uninfected mosquitoes	34

mF: microfilaria

A PCR-based test has been perfected for the identification of *Dirofilaria* sp. and for use in surveys for infected vectors of *D. immitis*[32]. The assay could amplify the target DNA fragment from free *D. immitis* larvae (both L3 and

microfilaria), individual infected mosquitoes, pools of 30 mosquitoes (including a single infected mosquito), infected mosquitoes stored under desiccation at room temperature for 7 months and whole blood of an infected dog. Field collected L3, identified tentatively as *D. immitis*, were confirmed by this assay. The primers did not amplify a monologous fragment from a mermithid or from 4 other species of filarioid nematodes (including another *Dirofilaria* species). There was no amplification using the PCR test for L3, identified tentatively as *Dirofilaria* sp. (possibly *D. tenuis*).

A highly repetitive DNA element from the genome of *D. repens* was cloned and sequenced[33]. The cloned repetitive sequence hybridized only to *D. repens* DNA, a single microfilaria in infected blood samples and a single L3 larva in mosquitoes.

A PCR assay based on the *Hha* I repeat was developed for the detection of *Brugia* in blood samples and mosquitoes. This assay has been used to detect individual L3 larvae. The sensitivity of this system is so great, that it may be possible to detect the L3 in 100 mosquitoes[34].

The major advantage of PCR system in the assays is that results are obtained after PCR by simple running the products on an agarose gel, staining with ethidium bromide, and looking for a 188 base pair band. Results are obtained very rapidly and the cost is comparable to conventional methods.

Conclusion

The available information indicates that the molecular biological techniques can successfully be employed for the accurate identification of genetically distinct members of some species complexes and insecticide resistant strains of mosquitoes and for the detection of infection and/or infectivity in mosquitoes due to viruses, and malarial and filarial parasites. However, at present, these techniques cannot be handled by field level workers and have to be carried out in centralised laboratories. With the rapid strides being made in the area of molecular biology, it is possible that simple and cost-effective field kits for conducting these assays could be made available in the not too distant future.

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ABSTRACTS

Some Research Projects Completed Recently

Critical evaluation of laser therapy in management of diabetic retinopathy :

The study was undertaken to assess the relative efficacy of argon green (514 nm) versus krypton red (647 nm) laser photocoagulation in the management of diabetic retinopathy and maculopathy respectively and to evaluate the efficacy of peripheral scatter laser photocoagulation in preproliferative diabetic retinopathy (PPDR).

The patients were categorised into different groups. Group 1 included 75 eyes of 68 diabetic patients with proliferative diabetic retinopathy (PDR) and high risk characteristics (HRC) while group 2included 60 eyes of 47 patients with PDR but without HRC. The high risk characteristics included new vessels on or within 1 disc diameter of the optic disc with and without vitreous or preretinal haemorrhage; new vessels on the optic disc (NVD) of approximately 1/4 to 1/3 disc area associated

with vitreous and/or preretinal haemorrhage; and new vessels elsewhere more than 1/2 disc area associated with vitreous and/or preretinal haemorrhage. All these eyes were prospectively randomised to treatment with argon green (38 and 30 eyes respectively in groups 1 and 2) or krypton red (37 and 30 eyes respectively) laser photocoagulation. At the end of one year follow up, there was no significant difference in the two groups treated with different wavelengths in causing regression of neovascularisation and improvement/stabilization of visual acuity.

Group 3 included 121 eyes of 88 diabetic patients with clinically significant macular oedema (CSME). The eyes were randomized to treatment with argon green (60) or krypton red (61) laser photocoagulation. At the end of one year follow up the eyes treated with both argan green or krypton red laser behaved similarly as regards the resolution of CSME and preserving/improving visual acuity from baseline levels.

Studies carried out on another group of 60 eyes (26 treated and 34 untreated of 42 patients) to evaluate the efficacy of peripheral scatter laser photocoagulation in PPDR demonstrated, that the number of eyes showing progression was much less in the treated group (34.6%) compared to control (55.9%). However, no conclusion could be drawn from these findings as the duration of follow up was too short (3 months) to comment upon the beneficial role of this treatment.

The results of the study suggest that the quality of care is not compromised by the availability of specific laser wavelength for fundus photocoagulation. However, a longer wavelength like krypton red offers the additional advantage of better penetration through hazy media and cataract and thus can be used with equal efficacy as the standard argon green laser in eyes with cataract and media opacities.

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Etiopathogenesis and management problems in pseudoexfoliation syndrome :

Patients with cataract (2792; 1695 males and 1097 females) and open angle glaucoma (147) were screened for pseudoexfoliation (PXF) and to find out how such eyes react to surgery for cataract and glaucoma and their management.

One hundred and sixty two of the 2792 (5.8%) patients with cataract had PXF (usually bilateral) with the highest incidence being found in the age group of 60-70 years. Incidence of PXF was more in males (61%) as compared to females (39%). There was an association of PXF with nuclear cataract, advanced stage of senile cataract and sublerxation of the cataractus lens. In patients with PXF extracapsular cataract extraction reduced the risk of vitreous loss and accidental capsule rupture, but poor mydriasis, zonular weakness and pigment dispersal may pose difficulties.

Surgery in PXF syndrome was often complicated by an intense postoperative iridocyclitis and sometimes by hyphaema and vitreous haemorrhage. It is felt that adequate surgical planning and gentle handling of tissues can prevent or reduce the complications.

Nearly one-fourth of all the patients with PXF had glaucoma. There was a strong correlation between the presence of PXF material in the eye and glaucomatous damage. In addition to pigment deposition in the trabecular meshwork, the accumulation of PAS-positive mucopolysaccharide-like PXF material in the outflow channels may be a causative factor in pseudoexfoliation glaucoma. Trabeculectomy yielded better results than medical therapy in PXF glaucoma though the results were worse than in primary open angle glaucoma (POAG). Hyphaema and uveitis were more commonly seen following trabeculectomy in PXF glaucoma than in POAG. Intraocular haemorrhage and uveitis might be due to the deposition of PXF material in the iris vessels and the resultant neovascularization seen as profuse dye leakage at the pupillary margin during iris fluorescein angiography. This test can be used as a sensitive and specific indicator of PXF syndrome in doubtful cases where even after full mydriasis, slit lamp biomicroscopy does not reveal the presence of PXF material.

Findings of the study thus suggest that an awareness of the PXF status will help in proper surgical planning, reduction of operative complications and identification of patients suspected to have glaucoma for long-term follow-up.

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ICMR NEWS

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

Meeting of the Task Force on Risk Factors for Acute Myocardial Infarction	January 27, 1997
Meeting of Experts and Investigators on Traditional Remedies for Diabetes Mellitus	January 27, 1997
Meeting of Project Review Committee on Virology and Microbiology	February 11, 1997

Participation of ICMR Scientists in Scientific Events:

Dr. Vasantha Thavraj, Senior Research Officer, Department of Paediatrics, All India Institute of Medical Sciences, New Delhi, participated in the VII International Congress on Anticancer Treatment at Paris (February 3-6, 1997).

ICMR AIDED SYMPOSIA/SEMINARS/WORKSHOPS/COURSES/CONFERENCES

Symposium/Seminar/Workshop/ Course/Conference	Date & Place	Contact Address
International Symposium on Sensorimotor Function of the Nervous System	January 31-February 1, 1997; (at Vellore)	Dr. M.S. Devanandan, Professor of Neurophysiology, Department of Neurological Sciences, Christian Medical College, Vellore-632004.
III National Seminar on Malaria and other Tropical Diseases	February 18-20, 1997; (at Bangalore)	Prof. N.J. Shetty, Organising Secretary of the Seminar, Centre for Applied Genetics, Bangalore University, Bangalore-560056.
V Brain Storming Session in Molecular Immunology	February 21-23, 1997, (at Varanasi)	Dr. Ajit Sodhi, Coordinator of the Session, School of Biotechnology, Banaras Hindu University, Varanasi-221005.
International Conference on Developing Human Relations and Ethnic Understanding	February 26-28, 1997; (at Hardwar)	Dr. S.K. Srivastava, Organising Secretary of the Conference, Department of Psychology, Gurukul Kangri University, Hardwar-249404.
Symposium on Current Trends in Research on Drugs of Herbal and Marine Origin	March 2, 1997; (at Gandhinagar)	Prof. N.S. Parmar, Principal and Professor of Pharmacology, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar-382023.
International Seminar on Frontiers in Surgical Pathology	March 3-5, 1997; (at Chennai)	Dr. Malathy Madhavan, Professor and Head, Department of Pathology, Dr. A.L. Mudaliar P.G. Institute of Basic Medical Sciences, Chennai-600113.

COUNCIL'S TRAINING PROGRAMMES FOR 1997-98

Leprosy

At the Central JALMA Institute for Leprosy, Agra:

- Training Course on National Leprosy Eradication Programme for Medical Officers (February 24-March 7 and September 15-26, 1997).

Virology

At the National Institute of Virology, Pune:

- Diploma in Medical Virology (July 1997-May 1998).

Reproductive Biology

At the Institute for Research in Reproduction, Mumbai:

- Training Course on Research Methods in Clinical Trials of Newer Contraceptives (April 14-25, 1997).
- Workshop on Genotoxicity (August 28-29, 1997).
- Workshop on Basic Molecular Biology for Clinicians (December 2-11, 1997).

Endocrinology

At the National Institute of Nutrition, Hyderabad:

- Annual Certificate Course on Endocrinological Techniques and their Applications (August 1-September 15, 1997).

Nutrition

At the National Institute of Nutrition, Hyderabad:

- M.Sc. in Applied Nutrition (June 1, 1997-February 28, 1998).

- Postgraduate Certificate Course in Nutrition (December 1, 1997-February 28, 1998).

Laboratory Animal Technology

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

- Training Course for Laboratory Animal Technicians (June 16-July 31, 1997).
- Training Course for Laboratory Animal Supervisors (September 1-November 29, 1997).

Occupational Health

At the National Institute of Occupational Health, Ahmedabad:

- Orientation Course on Occupational Health for Industrial Medical Officers (September 30-October 11, 1997).
- Training Course on Environmental Hygiene Practice (November 10-14, 1997).

ICMR PUBLICATIONS

	Price (Rs.)
Nutritive Value of Indian Foods (1985), by C. Gopalan, B.V. Ramasastri and S.C. Balasubramaniam, Revised and Updated (1989), by B.S. Narasinga Rao, K.C. Pant and Y.G. Deosthale (Reprinted 1995)	23.00
Growth & Physical Development of Indian Infants and Children (1972, Reprinted 1989)	10.00
Studies on Weaning & Supplementary Foods (1974, Reprinted 1996)	15.00
Studies on Pre-School Children (1974, Reprinted 1984)	6.00
A Manual of Nutrition (Second Edition 1974, Reprinted 1995)	6.00
Low Cost Nutritious Supplements (Second Edition 1975, Reprinted 1994)	4.00
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 & L.M. Rebello	10.00
Nutrition for Mother & Child (Third Edition 1978, Reprinted 1994) by P.S. Venkatachalam & L.M. Rebello	9.00
Japanese Encephalitis in India (Revised Edition 1980)	5.00
Some Therapeutic Diets (Fourth Edition 1988, Reprinted 1995) by Swaran Pasricha	4.50
Nutrient Requirements & Recommended Dietary Allowances for Indians (1990, Reprinted 1995)	16.00
Fruits (Second Edition 1996) by Indira Gopalan & M. Mohan Ram	20.00
Count What You Eat (1989, Reprinted 1994) by Swaran Pasricha	9.00
Diet & Diabetes (Second Edition 1993) by T.C. Raghuram, Swaran Pasricha & R.D. Sharma	18.00
Dietary Tips for the Elderly (1992) by Swaran Pasricha & B.V.S. Thimmayamma	3.50
Diet and Heart Disease (1994, Reprinted 1996) by Ghafoorunissa and Kamala Krishnaswamy	26.00
Depressive Disease (1986) by A. Venkoba Rao	58.00
*Medicinal Plants of India Vol.2 (1987)	136.00

* 25 per cent discount allowed to individuals.

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