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ELISA FOR THE DETECTION OF CELL MEMBRANE ANTIGENS OF SPERMATOZOA

It has been suggested that one of the causes of female infertility may be an antisperm antibody¹ and that conception may not take place if the iso-antibodies are present in high concentrations². It has also been suggested that sperm agglutinating activity may develop as a result of obstruction in efferent sperm transportation pathways. Spermatozoa from men with sperm agglutinating activity in the serum often show pronounced agglutination and exhibit reduced cervical mucus penetration. Studies have demonstrated that sperm agglutinating activity detected in both male and female is caused by sperm agglutinating antibodies³. There is therefore a need to develop simple, quick and sensitive methods for the detection of anti-sperm antibodies to help in the management of infertility.

The presence of antibodies to spermatozoa in unexplained infertility³ and following vasectomy⁴ can be detected by microsperm immobilization¹, passive haemagglutination², microsperm agglutination³ and microcytotoxicity tests⁵. The assays for detecting anti H-Y antibody include microcytotoxicity⁶, hybrid antibody method⁷ and haemagglutination test⁸. These conventional methods are time consuming, have an element of subjectivity and require fresh semen samples with good count and motility. An enzyme linked immunosorbent assay (ELISA) which is considered to be a quick and sensitive method was developed in the laboratory of the

Institute for Research in Reproduction (IRR), Bombay, to detect murine anti H-Y antibody and for screening mouse monoclonal antibodies directed to human spermatozoa. Phylogenetic conservation of H-Y antigen across the species has been reported⁹. Cross reactivity of this antigen using human and mouse spermatozoa was shown by IRR scientists by microcytotoxicity assay and ELISA¹⁰. It is for this reason that human spermatozoa were used as target cells to detect the activity of murine H-Y antibody in ELISA.

Preparation of H-Y Antibody

Anti H-Y antibody was raised in syngeneic Swiss mice by immunizing young females (8-10 wk old) intraperitoneally with male splenocytes (1×10^7) in PBS with complete Freund's adjuvant—CFA (1:10) four times, at weekly intervals¹¹. The ascites fluid was tapped and the activity of H-Y antibodies studied by cytotoxicity assay and ELISA and specificity by absorption with male and female spleen cells¹⁰.

Preparation of Monoclonal Antibodies Directed to Human Spermatozoa

Washed human spermatozoa obtained from normal healthy volunteers were used for immunization. BalbC mice were immunized with 1×10^6 washed spermatozoa in

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0.1 ml of PBS mixed with equal volume of CFA at fortnightly intervals. After four immunization the splenocytes of mouse which had a titer $\geq 1:3200$ was used for fusion. Fusion was carried out using mouse myeloma cells SP2/O using polyethylene glycol 1540 (J.T. Baker Chemical Co., Phillipsburg, NJ). Supernatants were screened for the presence of antibody using ELISA^{12,13}.

Preparation of Mouse Epididymal Sperm Samples

Epididymis from 3-4 mice were teased in PBS, pH 7.2 to release spermatozoa. The tissues were removed and sperm suspension was centrifuged at R.T. at 400 g for 20'. The cells were washed thrice and the cell count was adjusted to 1×10^7 /ml. Fifty microliters of this sperm suspension was added per well.

Preparation of Human Spermatozoa Samples

Semen samples with good count ($>68 \times 10^6$ /ml) and motility ($>50\%$) and negligible number of contaminating cells and without agglutination were used for these studies. Freshly collected semen samples were liquified at 37°C for 30 min and centrifuged at 400 g. The cells were washed thrice with PBS, pH 7.2 and used for coating plastic plates. Cell membrane antigens of spermatozoa were solubilized with 3 M KCl¹⁴. The protein concentration of solubilized antigen was determined by the method of Lowry and coworkers¹⁵ and used for ELISA.

Plastic Plates

Polyvinyl (Dynatech) and polystyrene plates from different sources (Greiner, Linbro, Falcon, Flow) were tested for their suitability for the assay.

Influence of Assay Conditions

Optimal conditions for the assay were determined by pretreatment of plates with PHA, fixing cellular antigens with glutaraldehyde and by the addition of BSA and Tween 20 to washing buffer (PBS, pH 7.2).

Procedure for ELISA

In experiments where phytohaemagglutinin (PHA) was used for pre-coating, 10 μ g of PHA in 100 μ l of PBS was added per well and incubated for 1h. The solution was taken out and wells were washed thrice. Washed mouse epididymal spermatozoa or washed human ejaculated spermatozoa were used for coating the plates. Fifty microliters of sperm suspension containing 5×10^5 spermatozoa was added per well. The uniform layer of sperm suspension was allowed to dry at room temperature. These cells were fixed with 100 μ l of 0.025 per cent glutaraldehyde for 30'. Plates were flicked to remove glutaraldehyde and washed four times, with PBS containing 0.05

per cent Tween 20 (polyoxyethylene sorbitant). Hundred microliters of appropriately diluted H-Y antibodies or 100 μ l of hybridoma culture supernatant was added per well and incubated for 1h, the supernatants were removed and the wells washed thrice. Rabbit anti mouse immunoglobulin raised, purified and conjugated to horse radish peroxidase at the IRR, Bombay by the method of Nakane and Kawaoi¹⁶ was used for this assay. The conjugate was titrated and a dilution of 1:800 was found suitable for the assay. Fifty microliters of diluted conjugate was added per well and incubated for 1h in dark at R.T. and the wells were washed four times. The substrate was prepared by mixing 4.86 ml of 0.1M citrate buffer, 5.14 ml of 0.2M Na_2HPO_4 buffer, 10 ml of distilled water, 8 mg of orthophenylene diamine and 50 μ l of 30 per cent H_2O_2 . Two hundred microliter of freshly prepared substrate was added per well. The reaction was stopped after 15' by the addition of 100 μ l of 4 N H_2SO_4 and the optical density was read at 492 nm in a titertek ELISA recorder.

The only difference when soluble antigen was used is that 10 μ g/well of antigen was added in carbonate-bicarbonate buffer of pH 9.6. The protein concentration can be reduced significantly when pure antigen is used for coating. Control ascites fluid and myeloma cell supernatants were used as negative controls. Antiserum of BalbC mice immunized with spermatozoa was used as a positive control. When the absorbance of the test sample was more than twice that of the negative control the test was considered as positive.

Titration of mouse anti H-Y antibody was carried out using both mouse epididymal spermatozoa and washed human ejaculated spermatozoa. The antibody was found to react with both mouse and human spermatozoa (Fig.1) confirming the high cross reactivity of this antigen. The titre of the antibody in ELISA was 1:160 whereas it was 1:32 by microcytotoxicity assay.

The results of titration of H-Y antibody carried out under different experimental conditions are presented in Fig.2. Washed human spermatozoa were used as the antigen in these studies. Control ascitic fluid was used as a negative control. It was seen that pretreatment of plates with PHA had no added advantage. Fixation of cells with glutaraldehyde and addition of Tween 20 and BSA to the washing buffer gave better and consistent results.

Microtitre plates of different makes such as polystyrene (PS; Greiner, Linbro, Falcon, Flow) polyvinyl chloride (PVC; Dynatech), were compared for their ability to bind sperm and the results are presented in Fig.3. Monoclonal antibody (Mab) to sperm antigen (F8) was used as the antibody, while washed immobilized human sperm

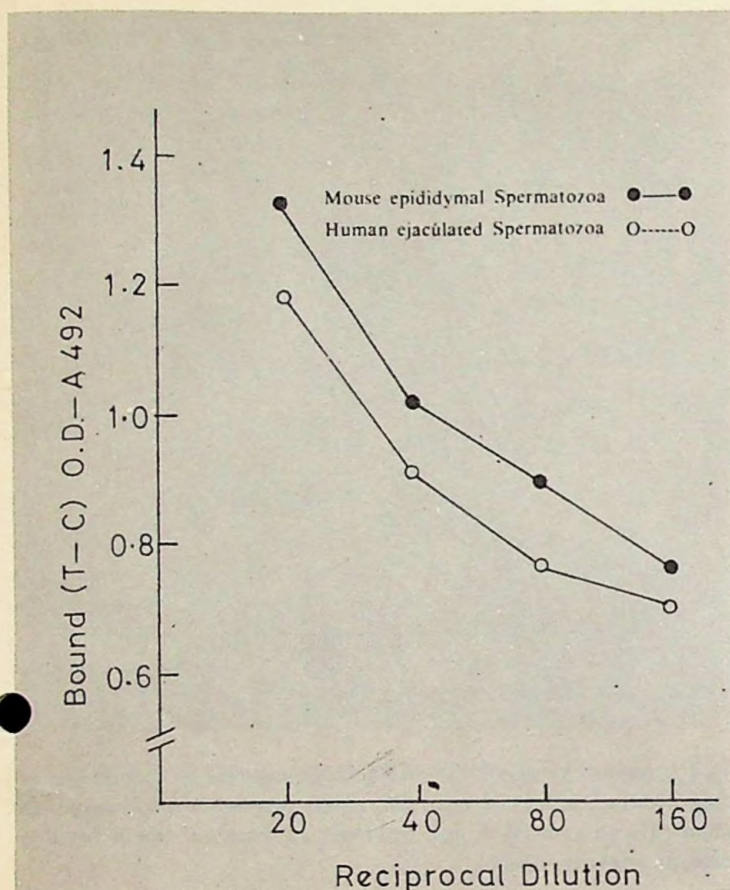


Fig.1 Titration of H-Y antibody using mouse epididymal spermatozoa and human ejaculated spermatozoa. Values represent differences between absorbance values (492 nm) for H-Y antibody and control ascites fluid. Each value is mean of triplicate values.

was used as the antigen. The PVC plates gave high background values as compared to PS plates. It was seen that reactions in the PS plates yielded higher absorbance values as compared to reactions in the PVC plates. Amongst the PS plates Linbro plates gave higher absorbance values. The other three brands of PS plates *viz* Falcon, Flow and Greiner were found suitable for this assay (Fig.3). The difference in these results was only marginal indicating that all brands of PS plates could be successfully employed for this assay.

Fig.4 shows the comparison of absorbance values when the antigen is presented in different forms, namely cellular and soluble. For this study 3 Mabs to human sperm were used. The immobilized cells (cellular antigen) gave higher readings with all the three Mabs tested. Myeloma supernatant from SP2/0 cell was used as the control.

The assays commonly used for screening hybridoma supernatant are RIA and ELISA. It is known that some of the monoclonals do not form suitable antigen antibody complexes as they are directed to a single epitope. Therefore, over the past few years ELISA has been the

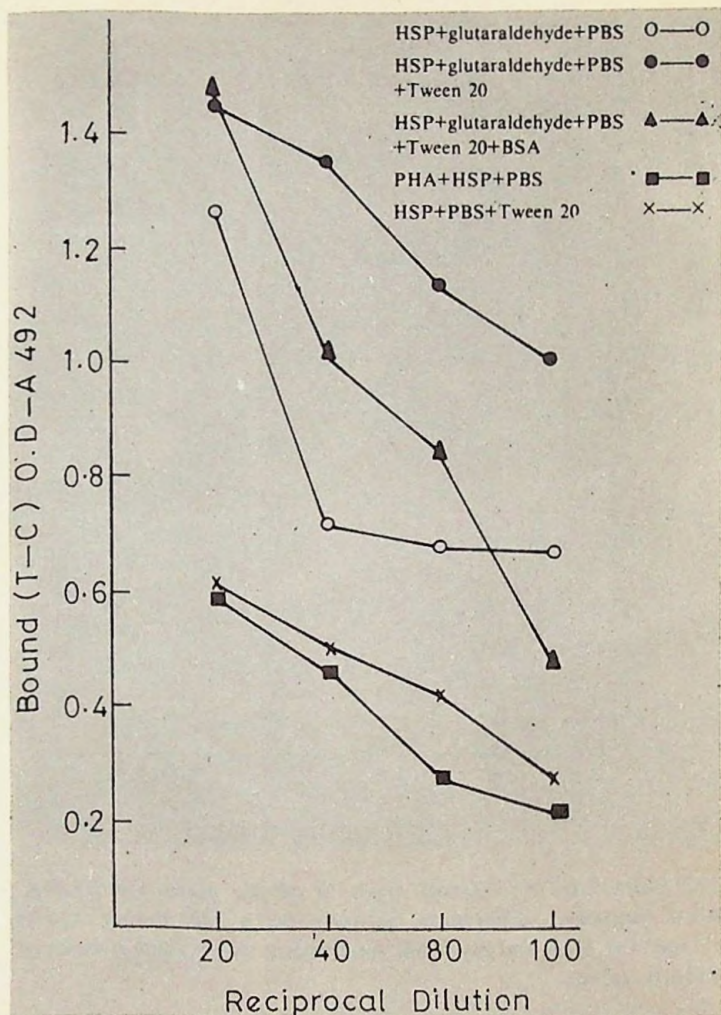


Fig.2 Titration of H-Y antibody under different experimental conditions. Human spermatozoa (HSP) was used as the antigen. Values represent differences between absorbance values (492 nm) for H-Y antibody and control ascites fluid. Each value is mean of triplicate values.

method of choice for screening antibodies directed to a wide variety of antigens.

Ever since the first report of Engvall and Perlmann¹⁷ and Van Weemen and Schuurs¹⁸, ELISA has been adapted and extensively used for various purposes and many assay methods have been developed. Compared to commonly used antisperm antibody assay^{1,3,19} ELISA technique has a number of advantages especially to screen a large number of hybridoma supernatants.

ELISA does not need excellent donor semen in terms of count and motility. It has been noted by scientists of IRR that coated microtitre plates could be stored at 4°C for several months without any loss of quality.

Sonicated membrane antigens of spermatozoa were used earlier at the IRR²⁰ for ELISA. A relatively mild method with 3M KCl resulted in high recoveries of membrane associated HLA antigens and has been shown to be superior to sonication¹⁴. In the present studies 3M KCl

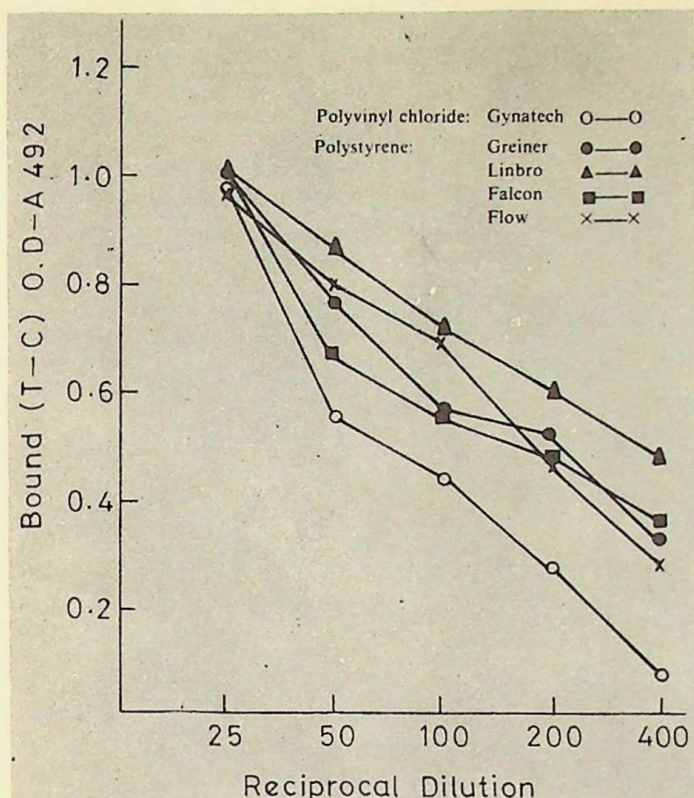


Fig.3 Suitability of different types of plastic plates for ELISA. Values represent differences between mean absorbance values (492 nm) for Mab and myeloma supernatant. Each value is mean of triplicate values.

was therefore used for solubilizing cell membrane antigens of spermatozoa. With soluble antigens the absorbance observed was low as compared to the cellular antigen and hence only cellular antigen was used subsequently. It was observed that precoating of plates with PHA had no added advantage and it was therefore not used in subsequent assay. The results obtained at IRR also indicate that low concentration of glutaraldehyde is convenient and suitable for fixing spermatozoa since it achieves adequate fixation and yields minimum background absorbance. Addition of Tween 20 to washing buffer gave reproducible results and increased the sensitivity of the assay and therefore this was used in subsequent assays. ELISA has been shown to be as sensitive and reproducible as any other binding assay¹⁸ and more sensitive than sperm agglutinating assay to detect antibodies to spermatozoa²¹.

ELISA was used for the detection of antibodies to H-Y antigen using mouse epididymal spermatozoa and human sperm. The reliability of ELISA to detect H-Y antigen was assessed by comparing it with microcytotoxicity assay and it was found that the former was more sensitive¹⁰.

These studies demonstrate that ELISA can be successfully employed for the detection of cell membrane

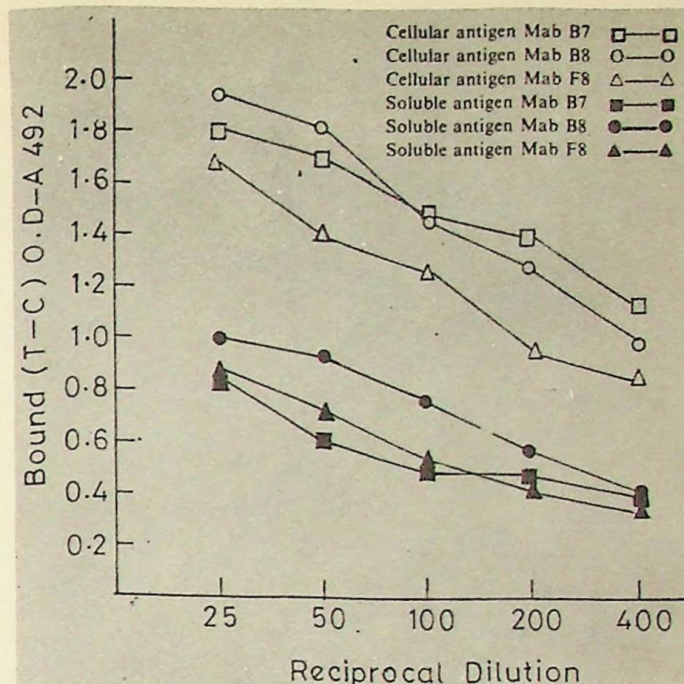


Fig.4 Comparison of cellular and soluble antigens of human sperm using 3 Mabs. Values represent differences between mean absorbance values (492 nm) for Mab and myeloma supernatant. Each value is mean of triplicate values.

antigens of spermatozoa using monoclonal and alloantibodies. It has also been shown that results may be influenced by the conditions of the assay. The ELISA is found to be more sensitive, and fast as compared to other conventional tests and is particularly suitable for screening large number of hybridoma supernatants. It is suggested that antibodies directed to spermatozoa and found positive by ELISA should also be assessed by other conventional methods.

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This write-up was contributed by S. Premchandran, S.V. Iyer, V.V. Khole and U.C. Hegde, Institute for Research in Reproduction, Bombay.

ABSTRACTS

Some Projects Completed Recently

Regulation of gene expression during cardiac hypertrophy.

The present investigation was carried out to examine the molecular signals triggered during development of cardiac hypertrophy and the stress mediated alterations brought about in the gene expression under this condition.

Cardiac hypertrophy was induced in experimental system by means of an operational procedure. The cytosolic proteins involved in the transport of RNA and stimulation of mitochondrial protein synthesis was purified 90 fold (molecular weight approximately 86,000).

The DEAE—cellulose column fractions having maximum effect on transport of RNA *in vitro* and on the enzyme activities were analysed by polyacrylamide gel electrophoresis (PAGE). The 7th and 8th fractions, having maximum transport activity as well as maximum alkaline phosphatase activity and Ca_2^+ dependent ATPase enzymes showed that 73,000, 68,000 and 43,000 molecular weight proteins were present in greater amounts as compared to other proteins.

The phosphorylation studies revealed that the nuclear envelope proteins of molecular weight 73,000,

68,000 and 43,000 which got phosphorylated, were induced by cytosol obtained from hypertrophic hearts. The peptide mapping analysis confirmed that these nuclear envelope proteins were the same which were present in cytosol and got transferred to the nuclear envelope after phosphorylation.

The *in vitro* transcription experiments revealed that nuclei obtained from the hypertrophic heart showed maximum ^3H -ATP incorporation into RNA as compared to the sham nuclei. Further, the maximum enhancement of transcription *in vitro* in isolated sham nuclei by 40 per cent hypertrophic cytosol suggested that the factors responsible for such stimulation of RNA synthesis may be induced during advanced stages of hypertrophy. Ammonium sulphate fractionation and DEAE-cellulose chromatographic separation of cytosolic components obtained from hypertrophic hearts helped in identification of the fractions containing factors(s) having the maximum stimulatory effect on *in vitro* transcription in isolated nuclei obtained from sham operated hearts. A 73,000 molecular weight protein was the most enriched protein having maximum stimulatory effect.

The phosphorylation studies of nucleoplasmic and cytosolic proteins revealed that the 73,000 molecular

weight protein was labelled at higher level in the cytosol obtained from hypertrophic heart and translocated to nucleoplasm when sham nuclei was incubated with labelled cytosol obtained from hypertrophic heart.

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Publications:

1. Kamala, J., Mariappan, M., and Rajamanickam, C. Changes associated with rat heart chromatin during cardiac hypertrophy. *J Biochem Intern* 13: 271, 1986.
2. Kamala, J., Mariappan, M. and Rajamanickam, C. Mitochondria functions during experimentally induced cardiac hypertrophy. *J Biosci* 10: 293, 1986.

Studies on perspectives of circulating immune complexes.

Serum samples from patients with bronchial asthma, myocardial infarction, systemic lupus erythematosus and acute glomerulonephritis were analysed for the presence of circulating immune complexes (CICs), to gain an insight into the clinical significance, origin, nature and biological functions of these moities.

CICs could be detected in 30 per cent of the 165 bronchial asthma patients. Of the 165 samples from patients phenotyped for C3 19 FS, 143 SS and 3 rare variants could be detected. Similarly, of the 327 samples from unrelated normal adults, in addition to common C3 phenotypes, 16 rare variants could be identified. Frequency of C3F gene was found to be 4.0.

CICs could be detected in 74 per cent of the 125 patients with myocardial infarction. When the patients were categorized in four groups representing the initial, acute, post, and recovery phases of infarction, the CICs could be detected in 70, 80, 56 and 90 per cent of the patients respectively. Levels of C3 persisted in the normal

range with higher marginal values in most acute phase patients.

Lipoproteins had a modulatory role on complement system, which is an integral part of the immune complexes. High and low density lipoprotein (HDL and LDL) activated the alternate pathway of the complement system in patients with myocardial infarction and thus could play a role in the pathogenesis of myocardial infarction/or evolution of infarct size. However, for the same lipoprotein concentration, degrees of activation by LDL was much higher than the activation caused by HDL. Very low density lipoprotein (VLDL) did not have any effect on C3 activation. CICs isolated from patients of myocardial infarction triggered spontaneous aggregation of normal human platelets.

Fourteen out of 17 patients of acute glomerulonephritis showed the presence of CICs. A higher level of CICs was found in sera of patients with systemic lupus erythematosus (558 μ g of AHG equivalent/ml) and with acute glomerulonephritis (787 μ g of AHG equivalent/ml). Glomerulonephritis patients showed a reduced number of C3b (CRI) receptor in the erythrocytes as compared to normal subjects. This is of high significance as CRI receptor is known to be involved in the clearance of immune complexes from the circulation and hence its estimation could be of diagnostic and prognostic importance.

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Publications:

1. Srivastava, N., Gupta, S.P. and Srivastava, L.M. Association between C3 complement types and bronchial asthma. *Hum Hered* 35: 263, 1985.
2. Das, N., Boro, N.S., Panchamoorthy, G. and Srivastava, L.M. Circulating immune complexes in myocardial infarction. *Indian J Med Res* 86: 74, 1987.

ICMR NEWS

Meetings of the following Scientific Advisory Committees/Project Advisory Committee/Project Review Committee of the Council were held during the month:

Meetings of the Scientific Advisory Committees (SACs):

SAC of the National Institute of Nutrition, Hyderabad	July 3, 1989 (at Hyderabad)
SAC of the Laboratory Animal Information Service Centre, Hyderabad	July 4, 1989 (at Hyderabad)

SAC of the Food and Drug Toxicology Research Centre, Hyderabad	July 4, 1989 (at Hyderabad)
SAC of the Institute of Cytology and Preventive Oncology, New Delhi	July 17, 1989 (at New Delhi)
SAC of the Centre for Advanced Research on Endomyocardial Fibrosis, Trivandrum	July 20-21, 1989 (at Trivandrum)
SAC of the Institute of Immunohaematology, Bombay	July 21, 1989 (at Bombay)

SAC of the Institute of Pathology,
New Delhi

July 26, 1989
(at New Delhi)

SAC of the Institute for Research
in Medical Statistics (Delhi
and Madras Chapters)

July 31, 1989
(at Madras)

Meetings of the Project Review Committee (PRC)/Project Advisory Committee (PAC):

PRC on Biochemistry and Cellular
and Molecular Biology

July 11, 1989
(at New Delhi)

PAC of the Assessment of the
Anti-tobacco Community
Education Programme

July 24-25, 1989
(at New Delhi)

Participation of ICMR Scientists in Scientific Events:

Dr. K. Banerjee, Director, National Institute of
Virology, Pune, participated in the WHO Intercountry

meeting on Review of Ongoing Studies on Non-A Non-B
Hepatitis, at New Delhi (July 10-13, 1989).

Dr. B.N. Saxena, Sr. Deputy Director-General,
ICMR, participated in the meeting of the Indo-US Joint
Working Group on the DBT-USAID Collaborative
Project on Contraceptive Development and Research in
Immunology, at Washington D.C. (July 11-14, 1989).

Dr. G.V. Satyavati, Sr. Deputy Director-General,
ICMR, participated in the IV World Conference on
Clinical Pharmacology and Therapeutics, at Mannheim/
Heidelberg, W. Germany (July 23-28, 1989).

Dr. Kamala Krishnaswamy, Deputy Director,
National Institute of Nutrition, Hyderabad, participated
in the Symposium on Drugs and Nutrition, held during
XIX International Congress of Paediatrics, at Paris (July
24-28, 1989).

ICMR AIDED SYMPOSIA/SEMINARS/WORKSHOPS/COURSES/CONFERENCES

Symposium/Seminar/Workshop/ Course/Conference	Date & Place	Contact Address
International Workshop on Women and Development: A Critical Appraisal of House- hold Level Research Methodologies.	July 2-11, 1989; Baroda	Dr. Amita Verma, Child Development Depart- ment, Faculty of Home Science, M.S. University of Baroda, Baroda.
I National Workshop on Enzyme Histo- chemistry and Immunohistochemistry.	July 5-22, 1989; Madras	Dr. M. Madhavan, Professor & Head, Department of Pathology, Dr. ALM Postgraduate Institute of Basic Medical Sciences, Madras.
Continuing Medical Education on Nuclear Medicine in Today's Practice.	July 15-16, 1989; Hyderabad	Dr. Kakarla Subbarao, Director, The Nizam's Institute of Medical Sciences, Hyderabad.
Symposium on Haemolytic Anaemias.	July 30, 1989; Hyderabad	Dr. G. Madhusudan Reddy, Medical Officer, Insti- tute of Genetics and Hospital for Genetic Diseases, Hyderabad.
National Conference on Recent Trends in Physical Chemistry.	August 2-3, 1989; Calcutta	Dr. R.V. Gopala Rao, Department of Chemistry, Jadavpur University, Calcutta.
Continuing Medical Education Programme on Rheumatology Update.	August 12-13, 1989; Hyderabad	Dr. Kakarla Subbarao, Director, The Nizam's Institute of Medical Sciences, Hyderabad.
VII IMA/CGP National Conference on General Practice.	August 18-20, 1989; Varanasi	Dr. S.P. Gupta, Organising Secretary 7th IMA/ CGP National Conference, IMA House, 7/31, Chetganj, Varanasi.
National Seminar on Strategies for Optimal Management of Cancer in Developing Countries.	August 24-27, 1989; Srinagar	Dr. Shad S. Akhter, Department of Medical Onco- logy, Sher-I-Kashmir Institute of Medical Sciences, Srinagar.
National Seminar on Newer Challenges in Medicine.	September 9-10, 1989; Jabalpur	Dr. B.N. Shrivastava, Professor and Head, Depart- ment of Medicine and Dean, Medical College, Jabalpur.

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Symposium/Seminar/Workshop/ Course/Conference	Date & Place	Contact Address
Seminar on New Trends in Methodology for Social and Population Research.	October 1-3, 1989; Tirupati	Prof. K. Mahadevan, Department of Population Studies, S.V. University, Tirupati.
National Workshop on Burns Injuries and their Sequele.	October 7-8, 1989; Chandigarh	Dr. C.P. Sawhney, Professor & Head, Department of Plastic Surgery, Postgraduate Institute of Medical Education and Research, Chandigarh.
XVI Annual Conference of Indian Immunology Society and Symposium on Tropical Immunology.	October 12-14, 1989; Sevagram	Dr. B.C. Harinath, Professor & Head, Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha.
XXII Annual Convention of the Indian Dietetic Association.	October 19-20, 1989; Hyderabad	Dr. Indira Ray, Reader, Home & Social Science Department, Calcutta University, Calcutta.
Brachytherapy Workshop for Cancer Cervix.	November, 1989; Betul (M.P.)	Dr. Anand Vijay Kumar, Secretary, Paramedical Section, Christian Medical Association of India, Smruti Theatre Compound, Nagpur.
All India Symposium on Biophysics of Cell Structure and Function.	November 6-8, 1989; Chandigarh	Dr. P.C. Mangal, Chairman, Department of Biophysics, Panjab University, Chandigarh.
Seminar on Zoonoses.	November 9-10, 1989; Dibrugarh	Dr. L.P. Datta, Director, Regional Medical Research Centre (ICMR), P.B. No. 105, Dibrugarh.
All India Symposium on Reproductive Biology and General Endocrinology.	November 26-28, 1989; Baroda	Prof. P.M. Ambedkar, Department of Zoology, M.S. University of Baroda, Baroda.
Workshop on Recent Advances in Intensive Care.	November 26-30, 1989; New Delhi	Dr. H.K. Kaul, Professor & Head, Department of Anaesthesiology, All India Institute of Medical Sciences, New Delhi.
XXXIII All India Obstetric and Gynaecological Congress.	December 28-30, 1989; Patna	Dr. J. Mishra, Department of Obstetrics and Gynaecology, Hospital for Women, Patna Medical College Hospital, Patna.
Seminar on Indian Speech, Language and Hearing Tests-the proposed ISHA battery.	February 8-9, 1990; New Delhi	Dr. S.K. Kacker, Professor & Head, Department of Otorhinolaryngology, All India Institute of Medical Sciences, New Delhi.

COUNCIL'S TRAINING PROGRAMMES

Leprosy

At the Central Jalma Institute for Leprosy, Agra:

- ... Training Course on Leprosy for Medical Officers (August/September, 1989).

Virology

At the National Institute of Virology, Pune:

- ... Short-term Course on Detection and Isolation of Arboviruses Using Mosquito Inoculation and Mosquito Cell Culture (October, 1989).

Endocrinology

At the National Institute of Nutrition, Hyderabad:

- ... Annual Training Course on Endocrinological Techniques and their Application (August/September 1989).

Nutrition

At the National Institute of Nutrition, Hyderabad:

- ... Annual Certificate Course in Nutrition (December 1, 1989 to February 28, 1990).

Occupational Health

At the National Institute of Occupational Health, Ahmedabad:

- ... Refresher Course for Industrial/ESIS Medical Officers (October 9-14, 1989).
- ... Training Course on Disaster Management of Hazardous Chemicals in Industry for Industrial Physicians, Chemists, Safety Officers, Hygienists (November 6-10, 1989).

Cytology

At the Institute of Cytology and Preventive Oncology, New Delhi:

- ... Indo-US Workshop on Chemoprevention of the Uterine Cervical Dysplasia with Nutrient Intervention (December, 1989).

Haematology

At the Institute of Immunohaematology, Bombay:

- ... Training Course in Blood Banking Technology and

Immunohaematology for Technicians (August 1-31, 1989).

- ... Training Course in Blood Banking Technology and Immunohaematology for Medical Officers (August 1 to September 30, 1989).

Laboratory Animal Technology

At the Laboratory Animal Information Service Centre, National Institute of Nutrition, Hyderabad:

- ... Training Course for Laboratory Animal Supervisors (September 1 to November 30, 1989).

Clinical Pharmacology

At the Seth G.S. Medical College, Bombay:

- ... Training Course in Clinical Pharmacology (November-December, 1989).

SOME AVAILABLE ICMR PUBLICATIONS

	Price Rs.
Nutrient Composition of Indian Foods (1989), by B.S. Narasinga Rao, K.C. Pant and Y.G. Deosthale	15.00
Growth & Physical Development of Indian Infants and Children (1972), Reprinted 1989	10.00
Studies on Weaning & Supplementary Foods (1974), Reprinted 1984	6.00
Studies on Pre-School Children (1974), Reprinted 1984	6.00
A Manual of Nutrition (1973), Second Edition 1974, Reprinted 1984	2.50
Low Cost Nutritious Supplements (1972), Second Edition 1975, Reprinted 1977	1.50
Menus for Low Cost Balanced Diets and School Lunch Programmes Suitable for South India (1959), Third Edition 1977, Reprinted 1984	2.00
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TOPICS FOR POSTGRADUATE MEDICAL THESES

In an attempt to improve the quality of research leading to theses submission by Postgraduate medical students, the ICMR proposes to compile and disseminate a list of researchable areas of national and scientific relevance as possible topics for theses. The list will also include suggestions regarding availability of necessary facilities and expertise for further guidance to the postgraduate students. In the first instance, the theses topics will cover 3 disciplines viz **Microbiology, Gynaecology & Obstetrics and Community Medicine**. It is hoped that these efforts will help in attracting suitable talent and in sensitization of medical students to biomedical research of high quality. Further, it is also hoped that the effort will also result in creation of a more conducive environment for fostering biomedical research. The Topics identified will be announced in the forthcoming issues of the ICMR Bulletin.

Indian Council of Medical Research Awards and Prizes 1989

The last date for submission of nominations/applications for the ICMR Awards and Prizes has been extended up to **September 15, 1989**.

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