



ICMR BULLETIN

DIRECTOR-GENERAL
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RESEARCH INFORMATION BULLETIN
DIVISION OF PUBLICATION & INFORMATION
ANSARI NAGAR NEW DELHI-110016.

EDITOR
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Vol. 8 No. 8

VIRUS RESEARCH (1977-78)

August 1978
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Koramangala
Bangalore-560034
India

Virus diseases like influenza, polio, hepatitis, encephalitis and dengue occur in major epidemics in our country. Many of these diseases exclusively affect agricultural labourers working in the forests, as in the case of Kyasanur Forest disease (KFD) in Karnataka. In other instances like Japanese encephalitis (JE), the disease is known to effect people living in villages in close proximity with pigs and fowl. There are no drugs effective against these virus diseases. Prophylactic control measures alone, based on a knowledge of the virus and virus vaccines can help in protecting human beings against these diseases.

The Virus Research Centre (VRC), Pune, which has been recognised as a premier centre in our country for research on arboviruses has now been renamed as National Institute of Virology (NIV), in view of its expanding activities in the field of virology. Since its inception the NIV has been carrying out major surveys in an effort to look for evidence of infection by viruses. These surveys have taken the NIV field teams to far flung areas of the country from Kashmir to Kerala and from Arunachal to Kutch. A large part of India has thus been mapped with regard to the existence of viral and rickettsial activity.

JAPANESE ENCEPHALITIS

Since the appearance of Japanese encephalitis (JE) in West Bengal in 1973, NIV has been continuously engaged in extensive studies in this area. During the year, a JE-like outbreak was reported from Chintamani taluk near Bangalore. The field station of the NIV at

Bangalore is currently investigating this outbreak.

Antibodies to JE virus are found extensively distributed in all parts of the country. While the role of pigs in the maintenance of the disease has been established, the probable role of birds and several species of mosquitoes in the natural cycle of JE virus has been receiving attention. Several strains of JE virus have been isolated from mosquitoes at Bankura. Studies have been carried out on the vectorial capacity of *C. bitaeniorhynchus* and *An. hyrcanus* mosquitoes. It was found that *C. bitaeniorhynchus* could transmit JE virus effectively. Though no transmission could be demonstrated by *An. hyrcanus*, the virus was found to persist in the mosquitoes upto 11 days after the infective feed.

Studies on birds have indicated that ardeid birds develop viraemia and a bird-mosquito-bird cycle has been demonstrated under experimental conditions, suggesting that ardeid birds may play a role in the natural cycle of JE virus. During the year 55 per cent of 196 wild ardeid birds caught in Andhra Pradesh showed neutralizing antibodies to JE virus. Experimental infection in ardeid birds produced specific neutralizing antibodies to JE virus. Experimental studies on ducks are in progress. Pigeons were found to circulate high levels of JE virus suggesting that a large proportion of vector mosquitoes fed on them could be infected.

The possibility of parasite-virus interaction leading to the occurrence of a disease like JE has been under investigation at the



NIV for the past few years. During the year, experimental work was carried out in pigs by infecting them by the oral as well as the subcutaneous routes. It was found that JE virus could infect the pig through the oral route, particularly when the pigs were also infected with parasites. Unlike in mice, there was no significant sickness or death in pigs either with the virus alone or in combination with helminths.

The ICMR Virus Research Unit at Calcutta, investigated an outbreak of JE which occurred in 1976, in collaboration with the NIV, Pune, and the Vector Control Research Centre, Pondicherry. In this outbreak a total of 277 clinical cases were reported. Specimens of acute blood (215), convalescent blood (76), CSF (90), postmortem brain (81), cattle sera (7), pig sera (47), goat sera (4) and 1475 mosquitoes from the affected area were received for investigation. JE virus could be isolated from a mosquito pool (*C. vishnui*) and a postmortem brain sample. Of the 76 paired sera tested by HI and CF tests, 33 showed a rise in titre against JE virus and 32 against Group B viruses while 11 did not show any definite rise in the titre. Of the 47 pig sera, 22 showed a high titre of HI antibody against JE virus. The cattle and goat sera were negative against JE antigen.

The Unit also continued studies on the production of a vaccine against JE using a local strain of virus in tissue culture.

KYASANUR FOREST DISEASE

Since the discovery of Kyasanur Forest disease (KFD) in Karnataka State by the Institute in 1957, the NIV has been responsible for monitoring the activity of the virus in the KFD area. Since 1975, although the Karnataka State Government has taken over the responsibility of surveillance of the activity of the KFD virus, the NIV continues to help the State's diagnostic laboratory in identifying the virus strains.

Improvement in the existing formalized KFD vaccine was attempted by using Roux bottle cultures of chick embryo fibroblasts and concentrating the virus by precipitating it by polyethylene glycol and by using aluminium hydroxide gel as an adjuvant. Concentration of the virus from pools of mouse brains could also be obtained by treatment with polyethylene glycol.

Studies have been initiated on the preparation of a sub-unit KFD vaccine. Brains

from infected mice are being utilised for this purpose, employing SDS, -2-mercapto ethanol, tween 80 and ether and triton X-100 for breaking up the virus. Studies on mice with sub-unit vaccine have indicated the possibility of developing such a vaccine for use in human beings.

Efforts to develop an attenuated KFD vaccine were continued. A strain of KFD virus was passed in primary chick cell cultures. Comparison between titres of the virus in the mouse brain, for the candidate strain for KFD vaccine and the wild strain indicated that while the candidate strain multiplied, it did not develop any CF antigen. Further studies are in progress.

Studies on the role of birds in the maintenance and spread of KFD virus have yielded interesting results. A total of 127 serum samples from 10 migrating and 7 resident species of birds from the Bharatpur bird sanctuary collected in 1973 were tested for antibodies to several Group A and Group B viruses as well as Congo and Batai viruses. Fifteen sera from four species of migratory birds were positive for HI antibodies to one of the Group A or B arboviruses. Only one showed HI antibodies to KFD while six had 'N' antibodies to KFD virus.

Studies on tick vectors of KFD were continued. At least 15 species of ticks are known to transmit KFD virus in nature. One of them is *Haemaphysalis papuana kinneari*. Life history of this tick in the laboratory was studied during the year. Studies were continued on the tick fauna, as related to their preference to vegetation, vertical distribution in the questing stages and the different hosts on which various stages of the ticks feed, and the size of the preferred host.

Colonization of *C. fuscocephalus* and *C. whitmorei* has been attempted in order to study their vectorial capacities for KFD virus.

Studies on tick repellence using 6 chemicals were carried out with 6 species of Ixodid ticks. A new method for the evaluation of repellent property of such chemicals has been worked out.

DENGUE

Dengue is known to occur throughout the country and all the four types of dengue virus

have been isolated. During the year, several cases of pyrexia of unknown origin in Pune were investigated by the NIV and 20 per cent of them turned out to be cases of dengue. NIV is also carrying out studies on the transmission of dengue by mosquitoes maintained on chicken and human blood. Studies carried out by the NIV on cases of acute paraplegia in collaboration with Ruby Hall Nursing Home, Pune, have indicated that dengue virus infection could be responsible for some of the cases of Guillain-Barre syndrome and acute transverse myelitis.

The pathogenesis of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), which are rare in India, has not been understood so far, in spite of large amount of work carried out in many parts of the world. It has been suggested that IgE-mediated release of histamine followed by increased vascular permeability may be responsible for the occurrence of DHF and DSS. IgE level was therefore determined in a few sera from the 1958 outbreak of DHF in Thailand, and IgE was found to be significantly higher in them compared to the controls.

The ICMR Virus Research Unit at Calcutta, has been actively engaged in surveillance of dengue, particularly DHF, in West Bengal. No frank case of DHF has so far been encountered. Of the 143 cases of pyrexia of unknown origin studied, in four dengue virus could be isolated. Serological survey for arbovirus antibodies in different parts of West Bengal were continued.

Preliminary experiments undertaken by the Virus Research Unit, Calcutta, to produce features similar to human DHF in mice, using dengue virus type 1, as the initial infecting virus, and adult adapted dengue virus type 2(AD-2) for secondary infection have yielded promising results. Further studies are in progress to investigate if passive transfer of pre-formed antibodies produces the same picture as previous infection.

Studies are also being carried out by the NIV, Pune, on the mosquito vector of dengue i.e. *Aedes aegypti*, in Maharashtra State. As a first step in the study of strain variation in *Ae. aegypti*, a strain maintained in the NIV colony has been screened by MacClelland's graded series of abdominal variants. Further screening is in progress.

The use of insect growth regulators has been suggested as one of the many methods for

control of mosquitoes. The NIV is now testing, in collaboration with the National Chemical Laboratory, Pune, a series of compounds against the fourth instar larvae of *Ae. aegypti*.

WANOWRIE VIRUS

Wanowrie virus is one of the new viruses isolated by the NIV from *Hyalomma marginatum isaaci* collected from sheep near Pune. There is report of only one fatal case caused by this virus.

During the year, experimental studies were carried out on Wanowrie virus infection in adult and infant mice. Virus titrations were carried out on various tissues and organs of experimentally infected mice during the first few days post-inoculation (PI). Virus titres varied in different tissues. Lesions in adult mice were confined to the liver showing marked vacuolation of the liver cells with small patchy areas of necrosis. The vacuolation was due to fatty change. There was proliferation of the endothelial cells lining the hepatic veins. Lungs also showed interstitial pneumonitis and haemorrhages. The small intestine of infant mice showed congestion and necrosis of the mucosa. No pathological lesions could be detected in the brain.

CRIMEAN HAEMORRHAGIC FEVER (CHF) - CONGO VIRUS

The NIV has been engaged during the last two years in extensive studies to determine the prevalence of the activity of CHF-Congo virus in the country. Intensive serological survey of human beings, cattle, sheep, goats, mules, rodents and birds is being conducted specially in the northern parts of India. A large number of ticks are also being processed for virus isolation.

GANJAM VIRUS

Studies on Ganjam virus were continued by the NIV. Pathological changes studied in inoculated mice indicated that the virus causes lesions in the liver, brain, lung and kidney of the inoculated mice.

The NIV investigated an "ill-defined" disease in local, exotic and cross-bred sheep in Palamer of Chittoor district, Andhra Pradesh. Acute phase serum samples from sheep and tick pools of *Haemaphysalis intermedia* yielded Ganjam virus. Sera were collected from local as well as exotic and cross-bred sheep. This is the first report

from India of a disease in sheep caused by Ganjam virus, which resembles the Nairobi sheep disease virus.

SEQUENTIAL IMMUNIZATION

Group B arboviruses *viz.* JE, KFD, dengue, West Nile, *etc.*, are widely prevalent in our country. Repeated infections are not unknown. Sequential infection by dengue viruses is alleged to be responsible for the occurrence of DHF/DSS. Individuals with antibodies to these viruses prior to infection may not sometimes show any antibody rise after vaccination. Further studies are required on the mechanism of cross-protection and cross-reaction and pathogenesis of Group B arbovirus infection. The NIV has been attempting to find out if sequential immunization with one or more viruses of this Group can offer protection against infection by a large number of Group B arboviruses. Whilst monkeys had been used in the earlier studies at the Institute, currently rabbits are being used. Four doses of the viruses in various permutations and combinations of JE, WN, dengue-2, and KFD (attenuated, killed and virulent) viruses have been employed. The results of this study are now being analysed.

Studies are also being carried out on IgG and IgM antibodies and their sequential development in infected rabbits and cross-reaction between these two antibodies. Studies are in progress to detect circulating immune complexes in rabbits sequentially immunized with different flaviviruses.

SANDFLY FEVER

Sandfly fever is known to have reappeared in India. Both the Naples and Sicilian strains of the virus were identified by the NIV in an outbreak near Aurangabad some years ago. Attempts have been made to plaque this virus in LLC MK-2, Vero and PS cell lines using Linbro plates. It was demonstrated that phlebotomus fever virus could grow in monkey kidney cultures with high titres but without producing any CPE while in PS cell line, the CPE was noted from the 4th day onwards. Attempts were made to produce haemagglutinin of phlebotomus fever virus. No haemagglutination was demonstrated by using tissue cultures procedures but infected mouse brains showed its presence on the 6th day after inoculation.

Many arboviruses are known to produce inhibition of growth of Rous' sarcoma. Phle-

botomus fever viruses were used in chicken and some inhibitory effect was noticed. Further studies are in progress.

The role of extra-human vertebrates in the natural cycle of phlebotomus fever virus is not fully understood. More than 20 species of laboratory-bred and wild-caught specimens have been tested and virus circulation could be detected in squirrels only. Hamsters bred in the laboratory were also found to yield peak titres.

Experimental studies in the laboratory on the role of *P. papatasi* have been undertaken to understand the rate of engorgement, infection, transmission, persistence and multiplication of the Naples and Sicilian strains of phlebotomus fever virus.

CHICKENPOX

Attempts are being made to develop a chickenpox vaccine. As the virus could not be grown satisfactorily in Vero culture, addition of a steroid/DEAE dextran to the tissue culture is being tried.

HEPATITIS

The NIV investigated an outbreak of hepatitis in Nellore district of Andhra Pradesh in February, 1977. An interesting aspect of this outbreak was its simultaneous occurrence in 25 villages. The NIV studied 230 samples of sera for the presence of HBsAg and for the distribution of antibodies. HBsAg was detected in the urban as well as the rural areas.

Attempts have been made by the NIV to standardise IAHA for detection of HBsAg and also to find out a suitable human 'O' group donor for this purpose. Purification of hepatitis A virus from faecal material has also been attempted by initial extraction, concentration with polyethylene glycol, followed by passage through Sephadex 2-B column.

At the ICMR Virus Research Unit, Calcutta, blood samples from 492 healthy individuals and the same number of voluntary blood donors were examined for detection of Australia antigen. It was found that 2.8 per cent of the apparently normal healthy population and 4.1 per cent of the voluntary blood donors in Calcutta carry this antigen. The incidence appeared to be higher in male adults than in females. There was no significant difference in the percentage of positive carriers in different age groups. The Australia antigen incidence

in the normal population of Calcutta (2.8 per cent) seemed to be similar to that of Delhi (2.76 per cent), and Chandigarh (2.7 per cent).

POLIOMYELITIS

Comparative studies on the killed parenteral vaccine and live oral poliovaccine are being continued at the Department of Microbiology, All India Institute of Medical Sciences, New Delhi. The object of this study is to find out the method most suitable for immunizing Indian children against poliomyelitis. Attempts were made to investigate if the response to live poliovaccine given orally could be improved by (a) increasing the number of doses, (b) altering the interval between the doses, and (c) changing the time of the year for administration of the vaccine. Preliminary results suggest that increasing the number of doses might improve the response. Further studies are in progress.

Another study is in progress at the B.J. Medical College, Ahmedabad, to determine the schedule, number of doses and the quantity of poliovirus in each dose of oral poliovaccine in order to achieve an effective sero-conversion and 100 per cent protection against poliomyelitis in children.

A study was also carried out on the excretion of poliovirus in children after administration of oral poliovaccine. In 142 children from whom faecal specimens were collected and examined, 83 (58 per cent) excreted viruses in the stool after the first dose of oral poliovaccine, 48 (34 per cent) after the second dose, and 35 (25 per cent) after the third dose. About 24 per cent children did not excrete any virus in their stool after any dose of oral poliovaccine. Higher rate of excretion of the virus was found after oral poliovaccine administration in the age group 0-12 months, while the rate declined in the age group 13 to 36 months.

Poliomyelitis surveillance was continued at the Council's Enterovirus Unit at Bombay. During January - November 1977, 986 cases of paralytic poliomyelitis were recorded, 30 per cent of the total cases were recorded between May and August.

During the year, paralytic illness following history of oral poliovaccine administration was recorded in 206 cases. Of these, 131 cases were subjected to virological

studies and 98 were found to excrete an intestinal virus. Polio virus was detected in 90 cases, the predominant strain being type 2. A 60 day follow-up study was carried out in 539 cases of paralytic poliomyelitis to assess residual paralysis. It was found that while 11.5 per cent cases had no residual paralysis and 77 per cent showed partial recovery of paralysis, in about 12 per cent cases there was no recovery at all. During poliomyelitis surveillance, approximately 10 per cent of the non-vaccinated group of population was studied for virus excretion. Of the 67 cases, 59 were found to excrete intestinal viruses. In 52 cases, there was poliovirus excretion, type 2 being the predominant strain, in contrast to the earlier isolations.

The Enterovirus Unit at Bombay, has initiated a programme to test the potency of oral poliovaccine at the time of its actual use in the field. For this purpose, weekly samples have been collected from a number of immunization centres from different parts of the city. About 260 samples have so far been collected and tested. About 93 per cent of the field samples of oral poliovaccine have not shown any drop in the potency. The study is still in progress. The Unit is also assessing the long-term stability of the oral poliovaccine samples from a large number of vaccine lots stored at -20°C for a period of 2 years at the Haffkine Institute, Bombay. Approximately 6 per cent of the vaccine lots have shown significant drop of potency on prolonged storage.

In continuation of studies on inhibitors of poliovirus in human milk and faeces, the Unit has now started collecting samples of saliva from infants. The aim of this study is to assess if there are any poliovirus inhibitors in the saliva which may adversely influence the activity of the virus in oral poliovaccine. So far, 25 samples have been collected and tested. None of the samples has shown the presence of inhibitors.

In view of the disturbing seroconversion rates obtained from trivalent oral poliovaccine, the Enterovirus Unit at Bombay has planned to immunize children with monovalent oral poliovaccine. The schedule that is being followed is monovalent type 1 followed by type 3, followed by type 2, followed by trivalent vaccine. Of the 96 infants who have been subjected to this study so far, 24 have completed the full schedule of the immunization. Further studies are in progress.

INFLUENZA

The NIV has been engaged in studies to isolate influenza virus and survey the antibodies in human sera. Studies are also undertaken on animal and bird sera collected in large numbers at the Institute.

A number of throat swabs from suspected cases of influenza collected from the Pune Municipal Corporation dispensaries were processed and 17 haemagglutinating agents were isolated in 1976 and nine agents in 1977. Some of these strains were identified as being related to A/Victoria/3/75. Most of these strains were highly sensitive to non-specific inhibitors in chicken serum.

Studies on the antibodies in animals to influenza A virus were continued. During the year, more than 300 goat sera were tested and most of them were found to be negative. More than 100 cloacal swabs which were collected from pigeons from the Zoological garden in Pune have been processed for virus isolation.

RABIES

Rabies continues to be a serious problem in the country. The number of persons who undergo antirabies treatment is steadily increasing every year. The Council is supporting several investigations on rabies and the important aspects of the disease which are being studied at different centres in the country are epidemiology, diagnosis and improvement of the vaccine.

A study is in progress at the B.J. Medical College, Ahmedabad, to determine the incidence of carrier state among stray dogs. There was no evidence of a carrier state among the animals examined so far.

The immunoperoxidase test used in the diagnosis of rabies has the advantage of detecting the antibody with an ordinary microscope in contrast to the fluorescent antibody test. Studies at the Central Research Institute, Kasauli, have shown that the test is satisfactory in cases of brains with a high concentration of rabies virus but is not dependable when the virus concentration is low.

Conventional rabies vaccine contains a large amount of nerve tissue and the encephalitogenic factor present in the nerve tissue may lead to the occasional development of neuromuscular accident in treated persons.

The vaccine could be rendered relatively free of encephalitogenic factor if the brains of very young animals are used for the preparation of the vaccine. But the best method would be to prepare a vaccine with the virus grown in tissue culture.

Another method of purification that could be adopted is treatment with the fluorocarbon, arcton 113, which selectively precipitates the nerve tissue which contains the encephalitogenic factor. Studies at the Central Research Institute, Kasauli, have shown that by arcton treatment the protein content of the brain emulsion could be reduced to about a fourth, while the infectivity was only slightly reduced. The vaccine lots prepared from arcton treated infected brain suspensions were found to pass the potency test.

Another study employing arcton to reduce the nerve tissue protein was carried out at the National Institute of Communicable Diseases, Delhi. In this investigation the brain emulsion was sonicated to improve the virus titre and then treated with arcton. The vaccine lots prepared have passed the potency test.

RESPIRATORY SYNCYTIAL VIRUS

Respiratory syncytial virus (RSV) is an important virus causing acute respiratory illness. In children, RSV is known to cause acute bronchiolitis, bronchitis and bronchopneumonia whereas in adults it causes colds and minor respiratory disorders. At the Institute of Microbiology, Madras, throat-swabs from 400 patients in the age group 0-30 years, suffering from lower and upper respiratory infections, were inoculated into human amnion tissue cultures for the isolation of RSV. A total of 82 strains producing CPE characteristic of RSV were isolated, of which 75 were confirmed as RSV. Children up to 5 years of age were found to suffer from a variety of lower respiratory illness due to RSV, the most serious cases occurring in children below one year of age. After the 5th year, the incidence of recovery of RSV from serious lower respiratory disorders declined, although the rate of recovery of RSV from minor lower respiratory and upper respiratory infections was found to be on the increase. On screening the sera of 544 apparently normal individuals in the age group 0-50 years for CPF antibodies against this virus, 23 per cent of the population were found to have had past experience with the virus, the highest percentage (27.5 per cent) being seen in the age group 0-10 years.

HERPESVIRUS

In addition to the screening of the sera of oropharyngeal cancer patients, the ICMR Virus Unit at Ahmedabad carried out studies on patients of cervical cancer along with matched controls. After collecting epidemiological data, 438 blood samples were tested by IHA test with herpes virus type 2 antigen, while 59 sera were tested by complement fixation test, and 47 by neutralization. In nearly 80 per cent of the cases of cervical cancer, antibodies were found for herpes virus type 2 by IHA as compared to 35 per cent in the control group, giving a significant difference in the percentage positivity.

Immunological studies on herpes simplex virus have been initiated at the All India Institute of Medical Sciences, New Delhi, with an aim to compare IHA test and radioactive chromium (^{51}Cr) release test for their suitability in the study of the seroepidemiology of herpes simplex virus (HSV) infections. The two tests have now been standardised and studies are in progress to evaluate them in cases of cervical cancer, oral and genital herpes infections, and normal healthy controls.

CLINICAL VIROLOGY

At the Clinical Virology Unit of the NIV, Pune, 165 cases of pyrexia of unknown origin, suspected cases of encephalitis, poliomyelitis, chickenpox, viral myocarditis, rabies, etc. were investigated. Paired sera from 126 patients and a specimen of brain for autopsy were obtained. Nearly 20 per cent of the cases investigated were due to dengue. Influenza A virus infection was diagnosed in 8 cases and Ganjam virus was isolated in 5 patients. Haematological investigations showed a low platelet count in 4 patients with a probable diagnosis of dengue.

Of the 46 cases of Guillain - Barre syndrome and 35 cases of transverse myelitis studied, 40 and 31 cases respectively showed antibodies to one or more viruses.

High titres of antibodies were found against six Group B arboviruses, one against measles virus, and four against herpes virus. Of the 11 cases of Guillain - Barre syndrome, 9 were due to Group B viruses, one each due to measles and herpes virus. The possibility of the involvement of WN and dengue-2 has also been considered in some of these cases.

The NIV investigated the aetiology of a large epidemic of gastroenteritis in some areas of the Ratnagiri district of Maharashtra. In this outbreak, 4,000 cases of gastroenteritis/dysentery were reported, 50 of them being fatal. Bacteriological studies carried out elsewhere on 52 stool samples had revealed the prevalence of *Shigella* infection in about one-third of these cases. NIV tested 61 stool samples for rotavirus by counter-immuno-electrophoresis and electron microscopy. Seven of the 61 samples showed precipitation lines for rotavirus. Electronmicroscopic study, however, did not reveal any rotavirus particles, but showed coronavirus-like particles in 10 samples and enterovirus particles in one.

CHEMOTHERAPY OF VIRUS DISEASES

The NIV has initiated studies on chemotherapy of virus diseases including studies on interferon, in collaboration with the National Chemical Laboratory (NCL), Pune, and the Central Drug Research Institute (CDRI), Lucknow. 6-MFA, an interferon inducer, was isolated from *Aspergillus ochraceus* by CDRI, Lucknow. 6-MFA was more effective against viruses like West Nile and KFD and has found to be different from the well known interferon inducer Statalon (obtained from *Penicillium stoloniferum*). Synthetic chemotherapeutic agents supplied by the NCL, Pune, are also being tested against some viruses in the laboratory.

TISSUE CULTURE AND CELL BIOLOGY

Tissue culture and cell biology studies at the NIV are concerned with two major areas: firstly, studies are carried out on the effect of viruses on cell lines, cell organelles and organ cultures and secondly, efforts are made to characterise the normal and transformed cells.

Studies on the effect of viruses on chromosomes were continued with measles virus in human blood leucocyte cultures and hamster cell cultures. Chromosomal aberrations observed included chromatic breaks, gaps, fragmentation and pulverisation.

Tracheal organ cultures were prepared from reptiles, birds and mammals and several viruses, particularly Sendai, measles, chikungunya, picorna, myxo and arboviruses were tested. DNA viruses included vaccinia and herpes viruses. The results indicated that mammalian tracheal explants supported multiplication of vaccinia, herpes simplex type 1

and Sendai viruses by exhibiting ciliostasis and of Chandipura, West Nile, herpes simplex type 2, polio and sandfly viruses without any noticeable effect on the ciliary activity. JE and chikungunya viruses did not grow in these cultures. On the other hand, chick embryo tracheal culture supported multiplication of JE and chikungunya viruses. Interference phenomenon could be demonstrated in tracheal cultures by observing ciliostasis. Attempts were also made to locate the site of virus multiplication in tracheal organ cultures using autoradiographic techniques.

Studies were continued on organ cultures of liver and kidney. It was shown that both could support the growth of a number of viruses. Studies on characterisation of cells involving the behaviour of normal virus infected and transformed cells in the presence of Concanavallin A (Con A) were also carried out. The studies on the agglutinability of different experimental cell cultures with Con A were continued. Normal diploid cells did not agglutinate with Con A. But cells infected with viruses agglutinated before visible CPE was observed.

Chromosomes in insect cells were also studied *in vitro*. Studies have been carried out by autoradiographic technique on cell lines obtained from *Ae. albopictus*, *Ae. w-albus* and *Ae. novo albopictus*. Attempts have been made to understand banding patterns in chromosome formation. These chromosomes were also studied after infecting the cells with JE, WN and herpes simplex and measles viruses. Chromosome studies of the virus infected mosquito cell lines revealed that despiralization of the metaphase chromosome

was a major effect besides chromatid and chromosome breaks with JE and WN viruses. Other changes in chromosomes are under study.

Characterization of insect cell *in vitro* was attempted on a number of cell lines, specially *Ae. novo albopictus* and *Ae. vittatus* and *Ae. w-albus*. Studies have been carried out on the morphology and growth rate including estimation of cellular proteins. Histochemical studies specially with regard to esterases and lipids, microspectrophotometric studies on cellular DNA and enzyme patterns of these cell lines are also being carried out. It was possible to differentiate various mosquito cell lines by their characteristic LDH and MDH electrophoretic patterns.

The pioneering work of the NIV on Singh's *Ae. albopictus* cell lines and tick cell lines is well known. Cell lines have been attempted from mosquitoes (*C. bitaeniorhynchus*), sandflies, ticks and mites. Efforts are being made to establish new cell lines from trombiculid mites.

A new method of growing cells on the microscope slide by using stainless steel cylinders was tried. Blood macrophages were cultured from BCG vaccinated and non-vaccinated donors, to observe the effect of viruses on this cell line. Efforts have been made to develop new cell lines from embryonic kidney. A new approach for the study of viruses has been introduced by using chick embryo developing *in vitro*. Teratogenic effects of viruses produced in early chick embryo (at primitive streak stage) may provide a new tool for identification of viruses.

ABSTRACTS

Some Research Projects completed recently

A clinico-epidemiological study of occlusive peripheral vascular disease in Delhi

A total of 169 patients of occlusive peripheral vascular disease forming 0.4 per cent of the total, annual, surgical admission in the Irwin Hospital, were studied. Thromboangitis obliterans was the most frequent condition followed by arteriosclerosis obliterans.

Thromboangitis obliterans was observed in young males, below 49 years of age (88 per cent). Gangrene was the commonest symptom and

the disease involved both the lower and upper limbs. The infrapopliteal pulses were frequently absent. The cardiovascular system, electrocardiograms, funduscopic and biochemical examinations were normal, except for significantly raised blood sugar, serum cholesterol and serum triglyceride levels. 98 per cent were smokers, usually of *beedis*. Thromboangitis patients came from the lowest socio-economic class and were heavy workers, mainly farmers. Calorie and protein malnutrition was present. Trauma and migrating thrombophlebitis were infrequent.

Arteriosclerosis obliterans occurred in old people, 60 years of age (72 per cent) or more. 84 per cent were males and 16 per cent were females. Ulcers, with or without gangrene, were the commonest symptom. Upper limb involvement was infrequent. The femoral and femoropopliteal pulses were absent most frequently. Diabetes was present in 36 per cent, cardiovascular disease in 20 per cent, electrocardiographic changes in 25 per cent cases. 72 per cent were smokers, usually cigarettes alone or combined with *beedis*. Calorie and protein deficiency was less frequent than in patients with thromboangitis and these patients were largely sedentary workers.

Angiography and histopathological studies revealed 11 per cent thromboangitis patients having iliac and femoral artery obstructions. Blood sugar, serum cholesterol and serum triglyceride levels were significantly raised in thromboangitis but were still higher in arteriosclerosis.

Thromboangitis obliterans was commonest in patients from Uttar Pradesh (58 per cent) while 60 per cent arteriosclerotics and 70 per cent controls came from Delhi. Significantly more than a quarter (28 per cent) of the total patients were farmers. Thromboangitis obliterans occurred mainly in the poorest socioeconomic group.

19 per cent patients with embolism, Raynaud's disease, phlegmasia caerulea dolens, collagen diseases (rheumatoid arthritis, scleroderma and systemic lupus erythematosus), iatrogenic (trauma and drug arteritis), non-specific arteritis, thoracic outlet syndrome and questionable tubercular arteritis, in that order of frequency, were also studied, females predominated (57 per cent).

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Effect of hydrogen chloride on lung surfactant

The effect of hydrogen chloride on lung surfactant of rats was studied. Healthy male albino rats were exposed to hydrogen chloride - air mixture for different periods under dynamic atmosphere at an average concentration of 440 ppm. At the end of the exposure, the rats were sacrificed and the lung surfactant activity was assessed by measuring the dynamic

surface tension, drawing air pressure-volume curves and estimating the phospholipid content. Routine histopathological examination of the lung tissue was also done.

Exposure of the animals for 5 hours did not alter the surfactant activity. Exposure for brief periods (6 days at 5 hours/day) indicated a mild decrease in surfactant activity as shown by the increased minimum surface tension and reduced phospholipid content. This was found to be reversible. Two weeks exposure showed a definite decrease in surfactant activity with incomplete recovery, indicating that the changes are due to a cumulative effect on continued exposure. With 4 weeks exposure the lung surfactant activity was decreased to a greater extent. Maximum and minimum surface tensions were increased, and phospholipid content was decreased. Static recoil pressure on deflation of the lung increased and the retention of air at 0 cm. of water pressure reduced. Histopathological examination revealed moderate to severe inflammatory reaction of the bronchial wall and alveolar septa, extensive alveolar hemorrhages, moderate areas of collapse and in some cases with mild pulmonary oedema. Thus, the lung surfactant activity was reduced to a mild degree on exposure to hydrogen chloride for brief periods and to a greater extent due to the cumulative effect of prolonged exposure producing alveolar collapse and oedema.

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High tibial osteotomy in degenerative arthritis of knee

Evaluation of the role of high tibial osteotomy in the treatment of osteoarthritis knee was undertaken in 30 patients. It was observed that age had no bearing on the results though stage of the disease had. Results were good in 19 cases (63.33 per cent), fair in 7 (23.33 per cent) and poor in 4 (13.33 per cent). Relief of pain (75 to 100 per cent) was seen in 22 cases (73.33 per cent). Results were better in cases with varus deformity and medial compartment involvement. Correction and maintenance of the femorotibial angle had a definite bearing on the results of osteotomy. Best results were achieved if the angle was more than 10° valgus.

It was felt that this is a simple procedure, not requiring prolonged postoperative immobilisation thereby reducing the load on hospital beds; with early union of osteotomy, without any danger of restriction of movements and with good relief of pain.

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Observation of ether preserved homogenous bone grafts in human subjects

With a view to assess whether ether preserved homogenous bone grafts could act as substitutes for autogenous grafts and deep freeze grafts, this study was undertaken using ether preserved bone grafts in human beings, following various orthopaedic operations.

It was observed that in all the cases (21) studied absorption and consolidation of the bone graft occurred. This was seen earlier in cancellous bone than in cortical bone. Immunological response due to homogenous grafting was not observed in any of the cases.

The sterility of the graft was maintained by ether preservation, which did not alter qualitatively and quantitatively the alkaline and acid phosphatase contents of the bone in 24 hours; and histological pattern of the bone. Ether thus seems to be a suitable preservative for homogenous bone grafts and could be tried for establishing bone banks.

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ICMR NEWS

The Virus Research Centre, Pune, has been renamed as the National Institute on Virology on 1st August, 1978, in recognition of its expanding activities in the field of virology. The Scientific Advisory Committee of the Institute met in Pune on 1st and 2nd August, 1978.

Dr. S.C. Pal, Director, Cholera Research Centre, Calcutta, participated in the Nobel Symposium on 'Cholera and related diarrhoeas - molecular aspects of a global health problem' held at Stockholm, Sweden, from 6th to 11th August, 1978.

Dr. S.G. Srikantia, Director, National Institute of Nutrition, Hyderabad, participated

in the WHO consultative meeting on 'Development of methodologies and guidelines for training of PHC workers in nutrition' held at Geneva between 7th and 11th August, 1978.

Dr. C. Gopalan, as President of the International Union of Nutritional Sciences, participated in the XI International Congress of Nutrition held at Rio de Janeiro, Brazil, from 27th August to 1st September, 1978.

Dr. P.K. Rajagopalan, Deputy Director, Vector Control Research Centre, Pondicherry participated in the meeting of the Technical Review Group III of the WHO Special Programme for Research and Training in Tropical Diseases, held at Geneva between 28th August and 1st September, 1978.

PROGRAMME OF SCIENTIFIC LECTURES AT THE INDIAN COUNCIL OF MEDICAL RESEARCH, NEW DELHI

Date	Topic	Speaker
8.9.1978	Importance of enteroviruses in India (Dr. J.B. Shrivastav Memorial Lecture)	Dr. T. Jacob John, Vellore.
22.9.1978.	Immune deficit in lepromatous leprosy and its correction	Dr. Kunal Saha, New Delhi.

ICMR AIDED SYMPOSIA/SEMINARS/WORKSHOPS

A laboratory teaching workshop in Physiology was organised by the Department of Physiology, All India Institute of Medical Sciences, New Delhi, between 17th and 29th July, 1978.

A seminar on 'Care of a worker' was organised by the Indian Association of Occupational Health at Baroda on 22nd and 23rd July, 1978.

A symposium on 'Clinical pharmacokinetics' is being organised during the V Southern Regional Conference of the Indian Pharmacological Society at Pondicherry on 9th and 10th September, 1978.

Symposia on 'Agricultural and food biochemistry', 'Nutritional and clinical biochemistry' and 'Separation methods in biochemistry' are being organised by the Society of Biological Chemists of India at Delhi between 3rd and 5th October, 1978.

A symposium on 'Surgery of the internal auditory meatus' is being organised at the All India Institute of Medical Sciences, New Delhi, on 5th and 6th November, 1978.

A symposium on 'Brain pituitary adrenocortical inter-relationship' is being held at the Institute of Medical Sciences, Varanasi, between 6th and 8th November, 1978.

A workshop on 'New techniques in neurosciences with special reference to the neurophysiology of higher nervous activity' is being organised at the National Institute of Mental Health and Neurosciences, Bangalore, between 6th and 17th November, 1978.

A symposium on 'Recent developments in applied analytical chemistry' is being organised by the Institution of Chemists (India) at the Medical College, Calcutta, on 11th and 12th November, 1978.

A seminar on 'Engineering and health care' is being organised by the Instrument

Society of India at Mysore, in November, 1978.

A symposium on 'Population structure and human variation' is being organised during the International Congress of Anthropological and Ethnological Sciences at Bombay between 19th and 21st December, 1978.

Symposia on 'Isoenzymes in clinical biochemistry' and 'Hormonal analysis in clinical biochemistry' are being organised during the V Annual Conference of the Association of Clinical Biochemists of India at the Seth G.S. Medical College and K.E.M. Hospital, Bombay, between 27th and 30th December, 1978.

A symposium on 'Problems in drug therapy in National health programmes' is being organised during the XI Annual Conference of the Indian Pharmacological Society at the All India Institute of Medical Sciences, New Delhi, between 29th and 31st December, 1978.

A symposium on 'Indigenous drugs in clinical medicine: Problems and possibilities' is being organised during the XXIV Annual Conference of the Association of Physiologists and Pharmacologists of India at the RNT Medical College, Udaipur, in December, 1978.

A symposium on 'Host-parasite interaction' is being organised at the Department of Zoology, University of Madras, Madras, in December, 1978.

Symposia on 'Reproductive biology' and 'Nutrition' are being organised during the VII International Congress of the Primatological Society at the Indian Institute of Science, Bangalore, between 1st and 5th January, 1979.

A symposium on 'Mechanisms of aggressive behaviour' is being organised during the Winter School on Neurophysiological and Neurochemical Correlates of Behaviour at the All India Institute of Medical Sciences, New Delhi, between 19th February and 3rd March, 1979.

COUNCIL'S TRAINING PROGRAMMES FOR 1978

Leprosy

At the Central Jalma Institute for Leprosy, Agra.

Training course in Leprosy for medical officers: (December, 1978).

Virology:

At the Virus Research Centre, Poona.

- (1) Diploma course in Virology of Poona University: (July, 1978).
- (2) *Training course for Technicians for a period of one year.
- (3) Short-term course in Animal Cell Tissue Culture: (June, 1978).

Reproductive Biology:

At the Institute for Research in Reproduction, Bombay.

- (1) Training course in Reproductive Biology: (January, 1978 to March, 1978).
- (2) Radioimmunoassay of Protein and Steroid Hormones: (January, 1978 to March, 1978).
- (3) Training course in Genetics: (January 16, 1978 to February 15, 1978).
- (4) Training course in Basic Immunology: (February, 1978 to March, 1978).
- (5) Training course in Contraceptive Research and Reproductive Endocrinology: (March, 1978).

Nutrition and Endocrinology:

At the National Institute of Nutrition, Hyderabad.

- (1) M.Sc. (Applied Nutrition) course: (June, 1978 to February, 1979).
- (2) Annual certificate course in Endocrinological Techniques and their Application: (October, 1978 to November, 1978).
- (3) Annual certificate course in Nutrition: (December, 1978 to February, 1979).

Haematology:

At the Blood Group Reference Centre, Bombay.

- (1) A certificate course in Blood Banking Technology and Basic Haematology for medical officers: (February 1, 1978 to March 31, 1978).
- (2) Training course in Blood Banking Methodology for Technicians: (May 2 to 14, 1978).

Occupational Health:

At the National Institute of Occupational Health, Ahmedabad.

- (1) ICMR/WHO training course in Industrial Toxicology: (March 1978).
- (2) Refresher course in Occupational Health for E.S.I. medical staff: (September 1978).
- (3) Training course on Chemical Hazards in Chemical Industry organised jointly by the NIOH and the National Safety Council: (December 1978).

Clinical Pharmacology:

At the Department of Pharmacology, Seth G.S. Medical College, Bombay.

Training course in Clinical Pharmacology: (January 15, 1978 to February 28, 1978).

Biostatistics:

At the National Institute of Nutrition, Hyderabad.

Annual training course in Biostatistics for Statisticians: (November, 1978).

Laboratory Animal Technology:

- (1) At the Virus Research Centre, Poona.

Short-term course in Breeding and Maintenance of Laboratory Mouse Colonies: (March, 1978).

- (2) At the National Institute of Nutrition, Hyderabad.

Laboratory Animal Technicians Training Course: (August, 1978 to September, 1978).

COURSES FOR TALENT SEARCH SCHEME FELLOWS

Haematological Techniques:

- (1) At the Department of Pathology, Christian Medical College, Vellore: (February 28, 1978 to March 10, 1978).
- (2) At the Department of Haematology, Post-graduate Institute of Medical Education and Research, Chandigarh: (September, 1978).

Clinical Pharmacology:

At the Department of Pharmacology, Post-graduate Institute of Medical Education & Research, Chandigarh: (March 6 to 15, 1978).

Clinical Chemistry and Medical Instrumentation:

At the National Institute of Nutrition, Hyderabad: (July 10 to 16, 1978).

Use of Radioisotopes in Medicine:

At the National Institute of Nutrition, Hyderabad: (July 24 to August 2, 1978).

Cell-Biology and Cyto-Genetics:

At the Indian Institute of Science, Bangalore: (January 1 to 10, 1979).

*Principles and Methods in Medical Statistics:

At the Tuberculosis Chemotherapy Centre, Madras.

*Methods used in Diagnostic Virology:

At the Virus Research Centre, Poona.

*Dates to be announced later.

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Printed and Published by Shri Prem Chand for the Indian Council of Medical Research, New Delhi at the ICMR Offset Press, New Delhi.

R.N. 21813/71