Female cancer patient patterns change from 1990 to 1994 in Manipal and Western India: Cervical declines and breast increases

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Incidence of cervical cancer has declined from 1965 in Bombay, and from 1984 in Kerala; whereas the incidence of breast cancer has increased in Bombay women from 1978. As female literacy, health awareness, and socioeconomic status improve, women marry and bear fewer children later, reducing cervical cancer, but increasing risk of breast cancer. Dakshina Kannada district in Karnataka resembles Kerala in development and health indices for females. Manipal patients are mostly from north Kerala, DK, coastal Karnataka, and Goa. Trends of > 10,000 patients from 1990–94 in the Manipal Hospital Cancer Registry show that numbers of all-new ($p < 10^{-5}$) and new-plus-follow-up ($p = 10^{-4}$) cervical cancer patients declined since 1992. Cancer of breast, ovary and corpus uteri increased from 1990 to 1994. Cancer of cervix, breast and ovary are leading types each year. Female cancer patient patterns in Manipal Kasturba Hospital from 1990 to 1994 show that the incidence of breast cancer has increased and that of cervical declined as in Bombay, Trivandrum, and elsewhere. The implications are discussed.

PATTERNS of cancer change with development because risk factors simultaneously change. Incidence of cervical cancer declined in Bombay from 1965 (refs 1, 2) and in Kerala from 1984 (ref. 3). Incidence of breast cancer concurrently increased[1,2,4] as it did internationally[5,6]. Improved female literacy leads to better health awareness. More females start work, marry, and bear fewer children later, raising their socio-economic status and age at marriage, as it has in Maharashtra and Kerala[7]. This generally raises the age at first coitus/pregnancy, reducing risk of cervical cancer and increasing that of breast cancer.

Female health and development indices in Dakshina Kannada (DK) district and Kerala with which it is contiguous, are similar, and, with those for Goa, Meghalaya, and Mizoram are among the best in India. Most Manipal patients are from north Kerala, DK, coastal Karnataka, and Goa. As underdeveloped people modernize, breast cancer replaces cervical as the most common type. This has happened in Bombay. We therefore studied patterns of female cancer patients in Manipal from 1990 to 1994 and consider their implications.

Manipal is 65 km northeast of Mangalore in Dakshina Kannada, a coastal district in south-west Karnataka. The recently established Hospital Cancer Registry in Kasturba Hospital (KH) Manipal's cancer wing – the Shirdi SaiBaba Cancer Hospital and Research Centre – collects data in two phases as KH is a general hospital and to economize on time and effort to efficiently generate a unified database. Phase I abstracts cancer site–sex–age–followup data from a computerized medical record database of all cancer, noncancer, new, old, and follow-up patients. Details of Phase I patients' cancer diagnosis, stage, therapy and outcome are abstracted online in Phase II. Phase II and Phase I data interact and interlock.

Records of patients were computerized in the late 1980s, primarily for administrative and billing, not scientific,

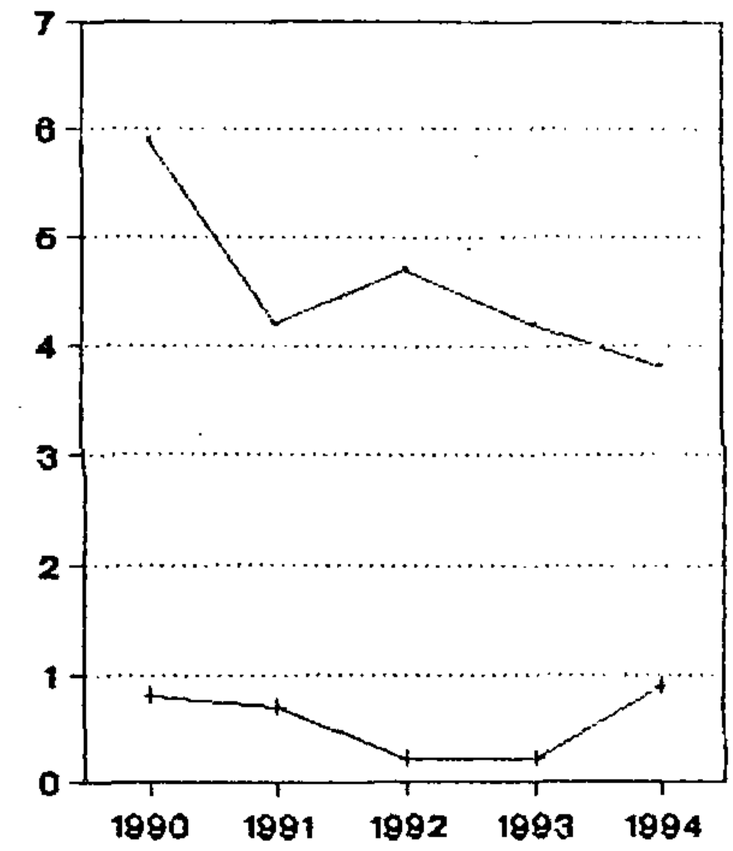

Figure 1. Kasturba Hospital cancer patient distribution (females), 1990–1994.

Table 1. All-new and follow-up female cancer patients, Kasturba Hospital, Manipal, 1990–94

Group description Year	Non-duplicate ca. pts *n*	Truly incident new ca. pts *n*	(%)	Follow-up pts *n*	(%)
1990	955	847	(88.69)	108	(11.3)
1991	1028	890	(86.58)	138	(13.4)
1992	1203	1159	(96.34)	44	(3.7)
1993	1209	1028	(85.02)	181	(14.97)
1994	1192	943	(79.11)	249	(20.89)
X^2 trend	n.d.	74.6		19.7	
p	–	$< 10^{-5}$		10^{-4}	

Table 2. Five major cancers in newly registered female cancer patients, Kasturba Hospital, Manipal (1990–94)

Year	Total *N*	Rank (1)	(2)	(3)	(4)	(5)
1990	847	Cx	Breast	Ovary	Stom	Thyroid
	n	201	92	43	38	37
	%	23.7	10.9	5.1	4.5	4.4
						Esoph
						36
						4.3
						Oth Mth
						36
						4.3
1991	890	Cx	Breast	Ovary	Oroph	Thyroid
	n	222	133	62	38	24
	%	24.9	14.9	7.0	4.3	2.7
					Esoph	Lung
	n				37	23
	%				4.2	2.6
1992	1159	Cx	Breast	Ovary	Oth Mth	Stom
	n	258	179	77	47	43
	%	22.3	15.4	6.6	4.1	3.7
					Esoph	
	n				46	
	%				4.0	
1993	1030	Cx	Breast	Ovary	Oth Mth	Esoph
	n	249	143	55	51	44
	%	24.2	13.9	5.3	4.9	4.3
						Stom
						42
						4.1
1994	943	Cx	Breast	Ovary	Esoph	Stom
	n	192	128	67	43	40
	%	20.4	13.6	7.1	4.6	4.2

Esoph, Esophagus; Stom, Stomach; Hypoph, Hypopharynx; Mth, Mouth; Cx, Cervix; Oth, other.

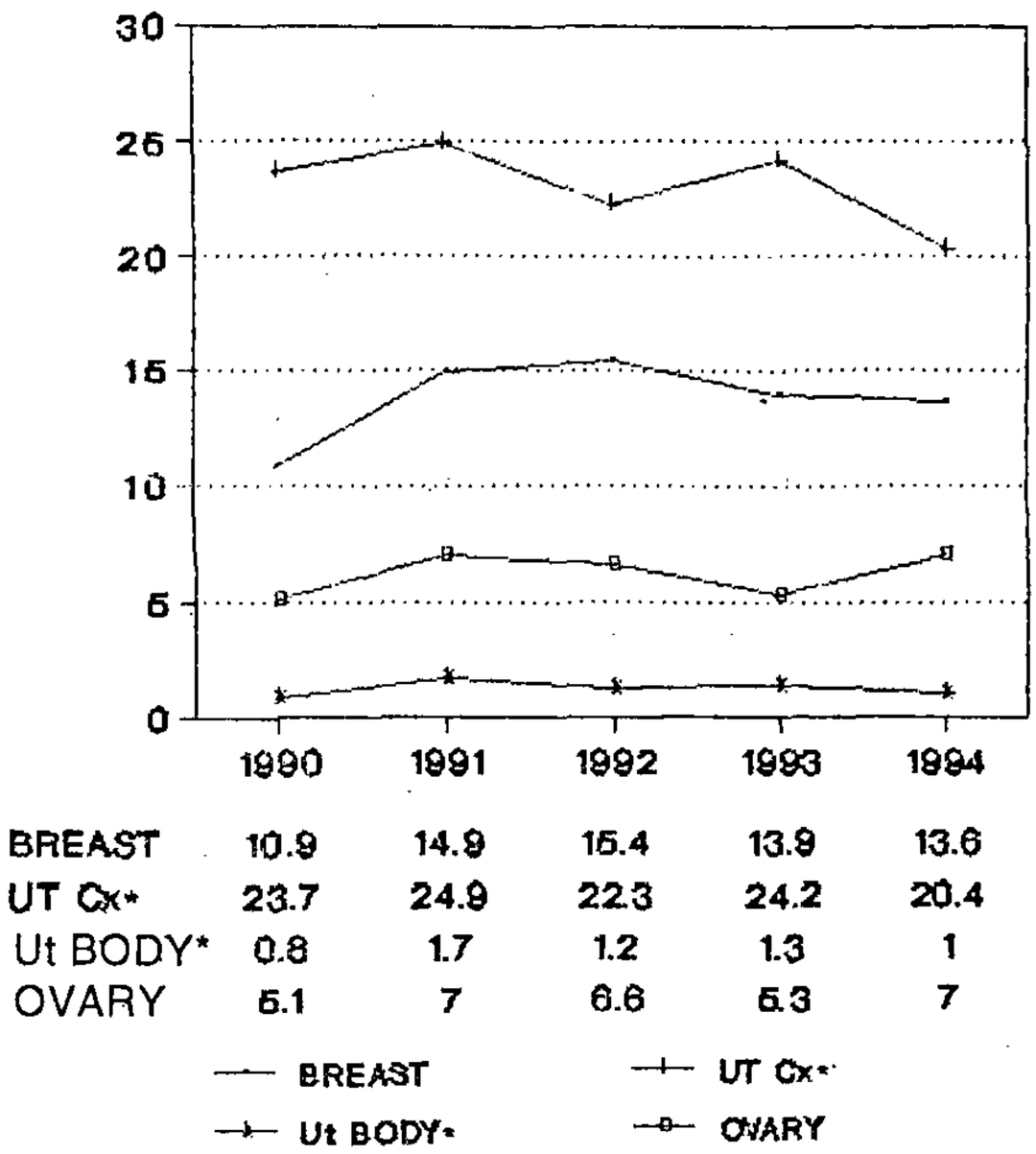

Figure 2. Data about female cancer patients, Kasturba Hospital, 1990–1994.

needs. Hence much new software was created to manipulate and organize this raw database into cancer patient data tables. Single entries of each cancer patient registered per year, and the lowest primary cancer code for each patient, were identified. A clean database of one entry per patient with one most correct cancer code was created. This task was arduous and slow because newly-written validated software was needed to remove annual duplicate or multiple patient entries and the multiple codes used by KH's coding system for primary and metastatic cancers. These data are internally consistent, validated for gender-cancer, primary-metastasis, primary, secondary and tertiary disease code differences and duplicate entries. Data were most reliable and abstractable after 1990 which was a cutoff year; pre-1990 cases were archived. Newly-diagnosed or truly incident, and follow-up cases were separated.

Male:female new and follow-up cancer patient ratios exceed 1 in Manipal (1.57), in the Bombay (Tata Hospital) and Trivandrum hospital registries (1.2 each) (see ref. 23) and in Bombay, Bhopal and Barsi populations (1.1 each) (see ref. 24)). The rest are mostly 0.8 to 0.9. Truly incident new female cancer ($p < 10^{-5}$) and follow-up ($p < 10^{-4}$) patients declined (Figure 1), as did all cancer patient percentages (Table 1). Cervical, breast and ovarian cancers lead. Esophagus, stomach, thyroid, 'other mouth', oropharynx, and lung cancer follow (Table 2). Cancer of breast, ovary and corpus uteri frequencies increase; that of cervical decline; vaginal and trophoblastic cancers are too few for analysis (Figure 2, Table 3). Manipal trends resemble those of Bombay, Trivandrum and Hong Kong (Table 4) though cancer of cervix remains 1. New patients exceed follow-up-plus new cervical patients but the trend is vice-versa for breast and ovarian cancer patients (Table 5). Age at marriage in Maharashtra and Kerala increases from 1951 (Figure 3).

Since these are hospital frequencies which are liable to selection bias, not rates from population-based data, and the data cover only five years, conclusions should be cautiously drawn. However, their consistency and resemblance to data from other Indian west coast places (Bombay and Trivandrum), and Hong Kong[8–10], Cali[11], Puerto Rico[12–15], Japan[12–15] and Singapore[25] suggest they may be real. Such changes also occur in disadvantaged groups of some developed countries, e.g. blacks in Bay Area, USA[12–15].

If confirmed, these findings suggest that changes in Bombay and Trivandrum also exist in Manipal and its

Table 3. Newly incident female cancers, Kasturba Hospital, Manipal, 1990–94

Cancer	Year	1990	1991	1992	1993	1994	Total 1990–94	1990–94 Mean annual	X^2
Total	*N*	847	890	1159	1030	943	4869	973.8	*P*
	%	100	100	100	100	100		100	
Breast	*n*	92	133	179	143	128	675	135	1.006
	%	10.9	14.9	15.4	13.9	13.6		13.9	0.31592
Ut Cx	*n*	201	222	258	249	192	1122	224.4	2.837
	%	23.7	24.9	22.3	24.2	20.4		23.04	0.09214
Ut Body	*n*	7	15	14	13	9	58	11.6	0.036
	%	0.8	1.7	1.2	1.3	1		1.2	0.84985
Ovary	*n*	43	62	77	55	67	304	60.8	1.203
	%	5.1	7.0	6.6	5.3	7		6.2	0.27276

Ut, uterine; Cx, cervix.

Table 4. Changes in females' cancers in Bombay, 1964–1993; Hong Kong, 1989–1991, Trivandrum, 1986–1991 and Manipal, 1990–1994

Place measure year	Type cancer Breast		Cervix	
Bombay-population				
Average annual age-adjusted incidence (AAIR)/10^5 (*n*) rank				
1964	20.4 (620)	2	24.9 (822)	1
1985	25.6	1	18.6	2
1988	25.0	1	21.0	2
1990	27.8	1	19.0	2
1993	30.1 (869)	1	19.1 (560)	2
p	<0.05*		<0.001	
*for 1965–85, 1982–1990, $p < 0.001$				
Hong Kong-population				
AAIR/10^5 (*n*) rank				
1989	34.0 (1038)	2	15.7 (475)	4
1990	34.6 (1097)	1	16.1 (500)	4
1991	34.6 (1106)	2	145 (459)	4
Manipal-Kasturba Hospital				
Newly incident % total (*n*) rank				
1990	10.9 (92)	2	23.7 (201)	1
1994	13.6 (128)2		20.4 (192)	1
p	n.s.		n.s.	
Trivandrum-Kerala				
% Total (*n*) rank				
1986	18.0 (352)	2	24.0 (468)	1
1989	21.2 (437)	2	22.6 (466)	1
1990	24.2 (597)	1	19.97 (492)	2
1991	22.4 (na)	1	19.1 (na)	2

Table 5. Major female cancers, Kasturba Hospital, Manipal, 1990–94

Cancer	Mean annual number and relative frequencies		
		Newly incident	Follow-up + Newly incident
Total	*N*	973.8	1118
	%	100	100
Breast	*n*	135	162.1
	%	13.9	14.5
Ut Cx*	*n*	224.4	254
	%	23.04	22.7
Ut body*	*n*	11.6	12.3
	%	1.2	1.1
Ovary	*n*	60.8	72.4
	%	6.2	6.5
Rest	*n*	542	619
	%	55.7	55.2

*Ut, Uterine; Cx, Cervix.

hinterland. Small differences and the few years covered by our data may explain the lack of statistical significance, though they concur with small increases elsewhere (see ref. 25). Cervical cancer decline may be due to later age at marriage and other factors like improved health awareness, cytology services, reduced exposure to risk factors and a cohort effect[1,2] for which Manipal data are not presently available. Incidence of breast cancer exceeded that of cervical in Bombay Parsis, Sindhis, Christians and Moslems mostly because of their higher literacy, educational, and socio-economic standards[16–19] even before it did of all Bombay women. The same factors may operate in Trivandrum, Kerala[3,23].

Females are less than males in India's population except in Kerala, DK, and 'developed' states like Goa, unlike in most other countries. Female cancer patients exceed males in some Indian registries[20–24] usually because cervical cancer dominates. Despite this, male patient excess in at least half the national cancer registries suggests that antifemale bias may reduce interaction of females with the healthcare system. Surprisingly, this also exists in Manipal, though it may indicate fewer cervical cancers.

New and follow-up patients of each cancer type peak in 1991–1992 in Manipal, maybe because of a cancer facility started in Mangalore that year. The many north

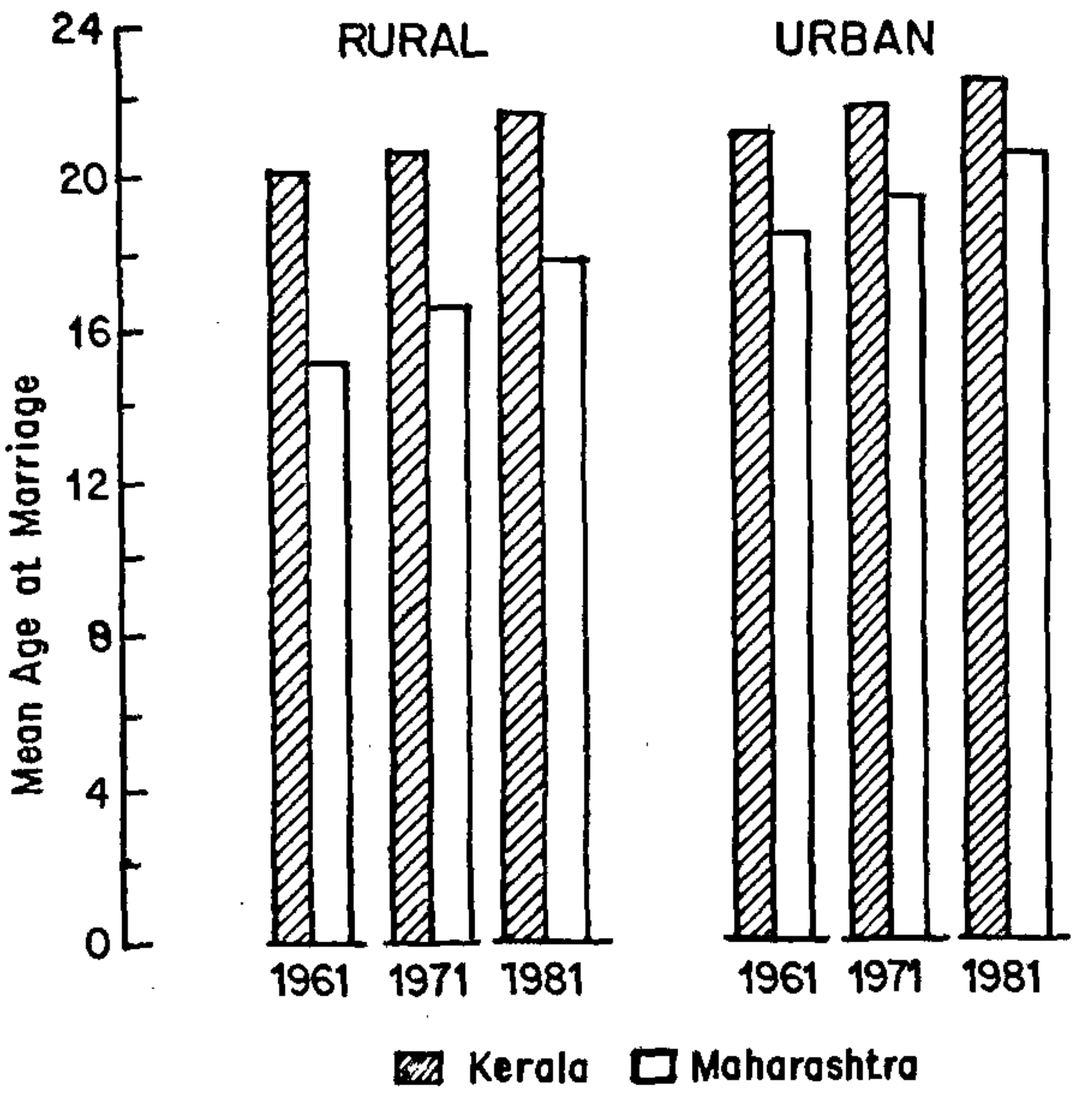

Figure 3. Trends in the level of mean age at marriage in Kerala and Maharashtra, 1961–1981.

patients from Kasargod, Kannur and Kozhikode may explain patterns resembling those of Trivandrum[3,20–23]. Differences in new and follow-up patients with these cancers may be due to increasingly available surgical treatment for breast and ovarian cancers and less due to availability of radiotherapy for cancer of cervix (though it exists in Kozhikode, and in Mangalore since 1992) outside Manipal. A similar trend was noted in Bombay[23]. Additional studies from places similar to Manipal like Goa, could confirm these findings.

Diseases presently uncommon in developing countries may increase in the future unless risk factors are reduced now[25,26]. How? Increase health education to improve health awareness, genital hygiene and safe sex, avoid unnecessary life-style changes with 'modernization' which are risk factors for such diseases[25]. If the epidemiological and biological risk factors for cervical cancer, common in developing countries, and of breast cancer which are opposite and more common with development of women[25–27], are known more widely, cervical cancer incidence may drop as in Sweden[28] and Kerala[29]. It may also help control births, and sexually transmitted diseases such as AIDS. Simultaneously, teach women about cancer avoiding diets and lifestyles, breast self-examination, the value of a regular cancer check-up after the age of thirty, and how to interact with the healthcare system without fear. Then the incidence and mortality of other major female cancers like breast, ovary, corpus uteri, may also decline. Knowing that these costly chronic diseases are preventable may empower women at risk with tools to avoid development becoming the mixed blessing it is in the affluent West, and live healthier lives. Cancer prevention education to reproductive age females may thus yield valuable social and economic dividends.

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Optimal pH conditions for the growth, release and stability of Japanese encephalitis virus in PS cell line

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Effects of pH of the medium on the growth, release and stability of Japanese encephalitis virus (JEV) were studied with an aim to optimize the conditions for the virus stock preparation. Eagle's minimum essential medium (MEM) was modified by addition of 30 mM HEPES and reduction of $NaHCO_3$ to 0.85 g/l. The pH values were adjusted to 7.2, 7.4, 7.6, 7.8 and 8.0 by 1.0 N HCl or 1.0 N NaOH. The study revealed that the optimum pH values for the growth, release and stability of JEV were 7.4, 7.8 and 7.6 respectively.

THE significance of optimum nutrient composition for the growth of Japanese encephalitis virus (JEV) in PS cell cultures has been reported earlier by us[1]. Further optimization of conditions for the release and the stability of the progeny virions is of utmost importance, especially if preparation of the vaccine is intended from this stock.

There are reports that among other factors, the viral integrity, stability and infectivity are dependent on the pH of the medium. Purified JEV loses its structural integrity at pH 6.2–6.6, the pH at which the haemagglutination test is carried out. Russel *et al.* found excellent preservation of infectivity of JEV during purification procedure using tris-saline EDTA buffer pH 8.2 (ref. 2). The stability of Dengue virus was found to be optimum at pH 8.0 (ref. 3). Fusion of virion envelope with the plasma membrane has been proposed as being involved in the infectious entry of enveloped viruses[4]. Fusion of viruses with liposomes containing phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and cholesterol was found to be pH-dependent, which may finally be accounted for the infectivity of the virus. Fusion of enveloped viruses with cellular or artificial lipid target membrane can occur either in neutral pH (Paramyxo and Herpes) or can be triggered by mildly acidic pH (Togaviridae, Rhabdoviridae, Bunyaviridae)[5]. Flavivirus infectivity and haemagglutinin are stable at pH 8.0 to 8.4 (ref. 6). It is also possible to achieve significant increase in amount of virus release per cell by increasing ionic strength of the medium[7].

With this background, the present study was carried out to know the effect of pH on the growth, release and stability of JEV (Nakayama strain).

PS cell line at passage number 79 through 87 was grown in Eagle's minimum essential medium [MEM (E)] supplemented with 10% goat serum (GS) and confluent monolayers were maintained in the same medium without GS. Subcultures were prepared by splitting the trypsinized monolayer in 1:4 ratio. Viable cell count was determined by Trypan blue dye exclusion method.

The medium M-8 (ref. 1) was used with a few modifications. HEPES buffer was added at a concentration of 30 mM and $NaHCO_3$ was reduced to 0.85 g/l. The pH values of the media were further adjusted by addition of 1.0 N HCl or 1.0 N NaOH. The test media having pH 7.2, 7.4, 7.6, 7.8 and 8.0 at 37°C were prepared.

JEV Nakayama strain which underwent 52 mouse brain passages and 5 PS cell culture passages was plaque-purified thrice in PS cells. The titre of the stock was $10^{7.5}$ PFU/ml.

Confluent monolayers of PS cells were employed for growth of virus. Milk dilution (MD) bottles of PS culture were infected with 0.1 PFU of virus/cell. Virus inoculum (0.4 ml) was allowed to adsorb on the monolayer for half an hour at 37°C. The monolayers were then washed with PBS, fed with test media having different pH and incubated at 37°C. The pH values were monitored visually using a phenol red pH indicator. Ten culture bottles were used to test each medium. Extracellular tissue culture fluids (ETCF) were collected at 24, 40 and 48 h post infection (hpi). The progeny viral content of pooled ETCF was assayed by end point dilution method and the 50% tissue culture infective dose ($TCID_{50}$) was calculated[8]. The experiment was repeated thrice and averages were compared using *t* test[9].

MD bottles were infected with JEV as described above and fed with M8 medium. ETCF of infected monolayers were discarded after 40 h post infection. Five groups of 10 MD bottles each were fed with the five test media having different pH and incubated for 1 h at 37°C. The virus released during 1 h was assayed by end point dilution method. Experiments were repeated thrice and averages were compared using *t* test.

Aliquots of the seed virus suspension having titre 7.5 log $TCID_{50}$/0.1 ml were diluted 1:10 to required pH val-