roc. Indian natn. Sci. Acad. B54 No. 4 pp. 215-238 (1988)

INSA–DR TIRUMURTI MEMORIAL LECTURE, 1987
History of Researches on Plague leading to Its eradication in India

S C SEAL, FNA
2 Fern Place, Calcutta 700 019

(Delivered on 16 November 1987)

THE PROBLEM OF PLAGUE—Historical aspect

From the evolutionary point of view diseases that were prevalent in the animal kingdom gradually involved human beings by legacy or contact as they started to live in groups or communities. Plague is one of them which even now gets from the animals (rodents). From the available historical accounts it appears that plague visited all regions and countries in the world at one time or the other since the middle ages. leaving a few islands and isolated area free during the course of known historical period.

The first human epidemic on record according to Wu-Lien-Tech et al. (1936) was the outbreak among the Philistines in 1320 B C. It was characterised by the appearance of emerods (buboes) in their secret parts, as described in I Samuel V and Vi in the Bible. The Indian scripture like the *Bhagabata Purana* (1500–600 B C) gives the disease an earlier antiquity by referring to deaths caused by an epidemic disease preceded by an epizootic among rats. Men were warned to quit their houses when the rat fell from the roof (apparently *R.rattus)*, jumped about as if it was drunk and died. As the time passed the accounts of plague became more reliable. It was prevalent in Egypt, Libya and Syria as found in the writings of Rufus, the physician at Ephesus round about 100 AD.

Of the classical pandemics the *First* one occurred during the region of the Emperor Justinian (AD 542). It started from Pelusium, the great trading centre of lower Egypt from where it spread through N Africa to Roman empire on the one side and to Syria, Palestine and Constantinople on the other and then to other parts of Europe and Asia reaching London in AD 662. It lasted for 50–60 years and killed 100 million people.

The *Second* pandemic started in the 14th century (AD 1347) from Caffa in Crimea and spread to China and India on the one side and Asia Minor and N Africa on the other. It was imported to Geneva through the Army and from there it appeared in other parts of Europe reaching England by AD 1349. The disease then ravaged Europe for well-nigh three centuries under the horror of what was known as "Black Deaths" taking a toll of 25 million human lives. Retrogression started in the 17th century from west to east completely leaving the European stronghold by 1841. During this time quarantine laws were first passed in 1374 by Count Barnardo of Reggio and by the Venetians in 1403, followed by land cordons and sanitary improvements in the town on the basis of the ideas that rats were involved and the spread was through the maritime transport. About this time Gregory of Tours (540–94 AD) convincingly traced the transportation of plague infection from Spain to Marseilles harbour in 588 AD in support of the view that the Justinian and Byzantine plague in 542 was brought by the corn-ship from Palestine.

The history of plague in India can be divided into two distinct phases namely, Phase I (before the discovery of plague bacillus) and Phase II (after the discovery of plague bacillus). During the first phase reference to plague is available in *Bhagabata Purana* (1500–600 BC) and next to it the occurrence of plague in Malabar in 1325 AD, in Malwa in 1403 AD and in Punjab in 1617 AD during the reign of the Moghul Emperor Jahangir. It was repeated in Kathiawar and Kutch in Gujarat in 1812–21 AD and followed by 'Pali' plague in Rajasthan. (1836–1838 AD)

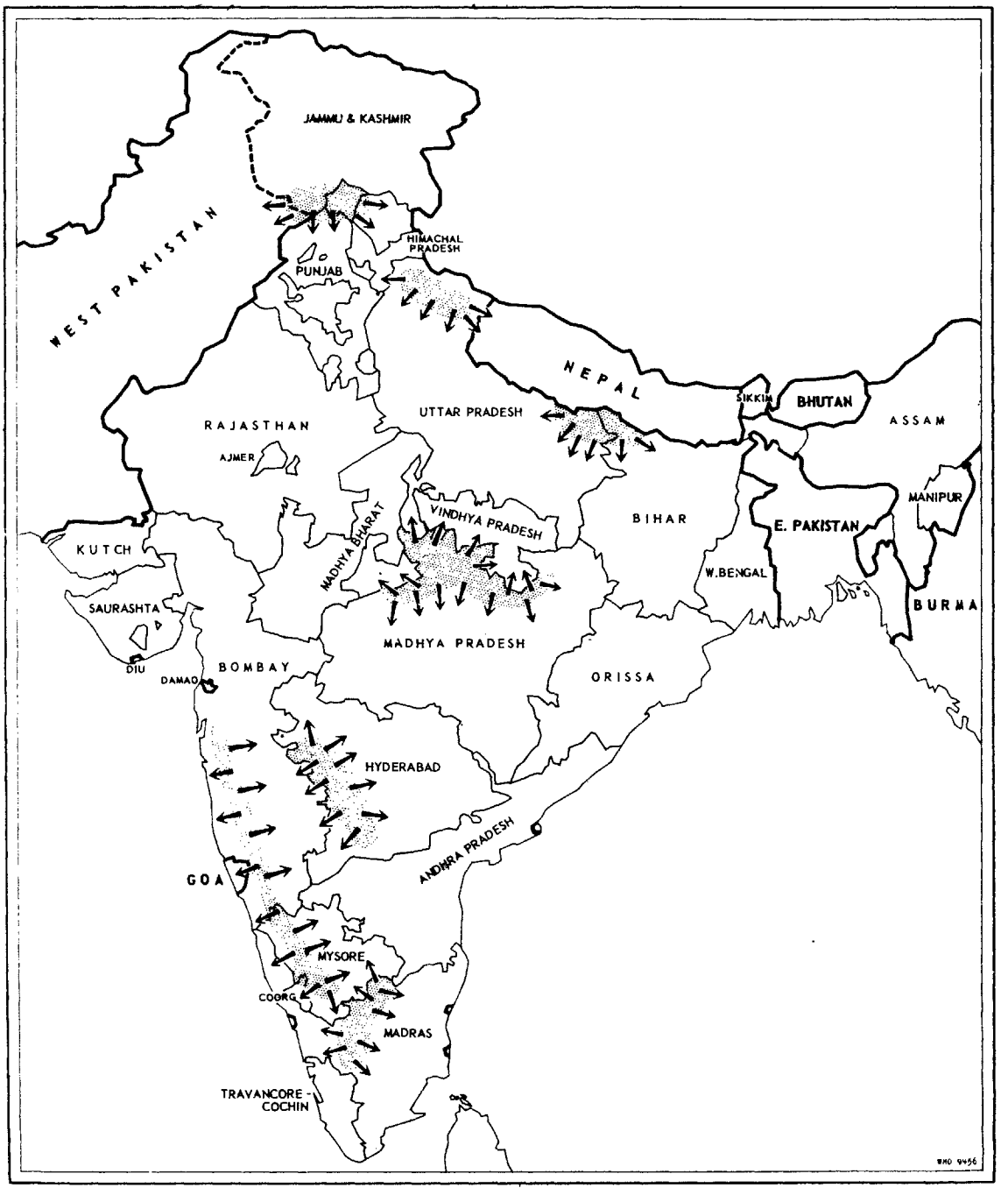


Figure 1 Endemic plague foci in India according to Sharif (1951). Arrows indicate only the direction of radiation of plague, not the actual course of its progress.

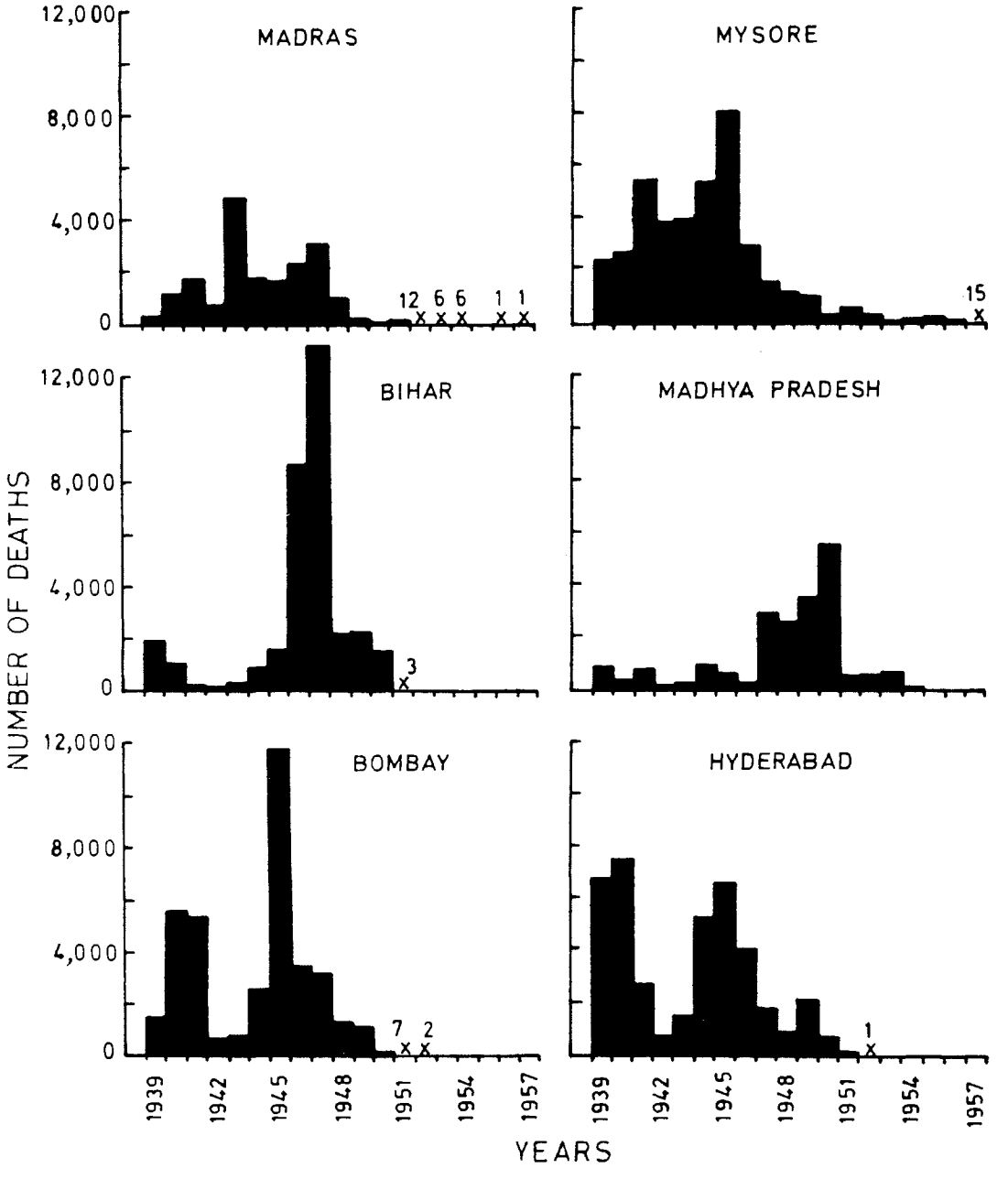
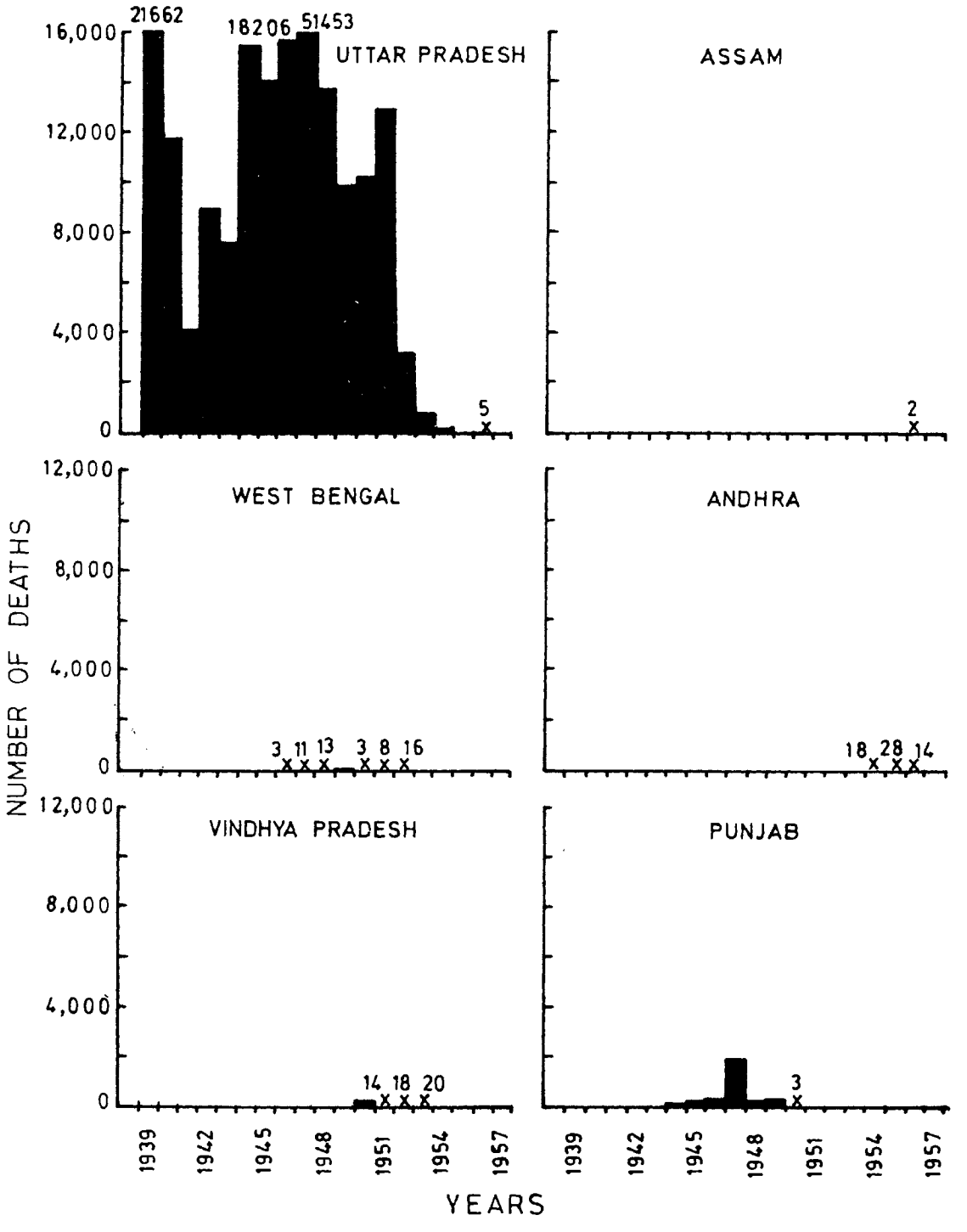


Figure 2 a-b Plague deaths in different States in India (1939–57)


UTTAR PRADESH
21662
18206
51453
5
ASSAM
2
WEST BENGAL
3 11 13 3 8 16
ANDHRA
18 28 14
VINDHYA PRADESH
14 18 20
PUNJAB
3
16,000
12,000
8,000
4,000
0
NUMBER OF DEATHS
1939
1942
1945
1948
1951
1954
1957
YEARS

The *third* Pandemic started in 1894 AD and was traced to the reappearance of plague in South China at Yun-nan-fu in 1866 whence it reached Hong Kong in 1894 AD and from there to Calcutta (India) in 1895 and Bombay in 1896 by the maritime route. Plague bacillus which was discovered by Yersin and Kitasato in 1894 was isolated on the 17th April in Bombay and 10th October at Calcutta in 1897. From these two cities the disease spread to almost all parts of the West and North but not towards Assam and East Bengal. The author during the course of his research discovered the reason why the disease did not invade these two regions. This epidemic situation prevailed till 1918 with a peak in 1907 and a total death record of 10.25 million. In 1907 itself there were 1,315,892 deaths. In 1910, a great epidemic of Pneumonic plague occurred but by 1919 all plague epidemics began to decline everywhere except Java and East Indies leaving certain persistent foci in India defined by Sharif (1951) (figure 1). It started with a decinnial mortality rate of 183.3 per 100,000 in 1898–1908 to 11.7 in 1929–38 and 0.02 in 1959–68 before its fang could be completely broken. Its ingress into different states in India (Seal 1960b) is shown in figure 2, and table 1.

General Characteristics of the Epidemic Waves

A typical epidemic wave is generally symmetrical differing with normal curve in regard to the height of the peak. In the pre-epidemic periods it takes some time to develop epizootic among the field and sewer

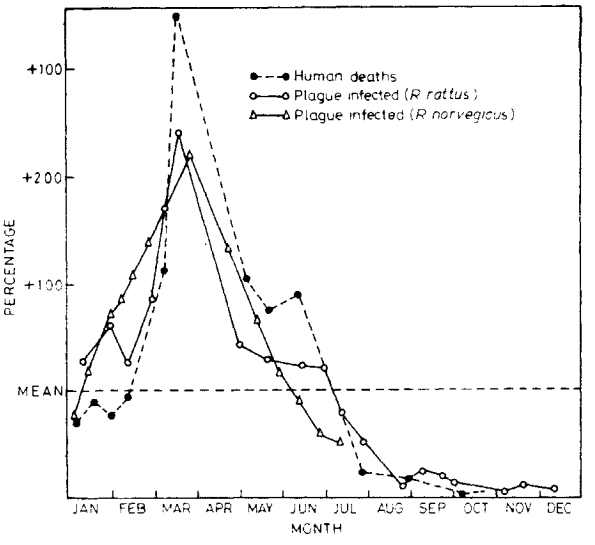


Figure 3

Table 1 *The province-wise distribution of plague mortality between July 1898 and June 1932*

Province	Mean population Census 1901, 1921 and 1931	Total plague deaths (July 1898–June 1932)	% of All India total	Mortality per 1000 of mean population
Punjab	21,142,793	3,489,123	28.7	165.0
Bombay	19,877,756	2,460,132	20.2	123.8
U.P.	47,164,594	2,911,837	23.9	61.7
Bihar + Orissa	34,692,676	1,113,937	9.2	32.1
C.P.	13,991,863	468,165	3.8	33.5
Hyderabad	12,855,934	425,302	3.5	33.0
Mysore	5,970,446	314,673	2.6	52.7
Rajputana	10,330,957	282,312	2.3	27.8
Madras	42,168,483	227,184	1.9	5.4
C.I. Agency	7,653,893	149,941	1.2	19.6
Burma	5,970,446	149,427	1.2	11.8
Other areas	38,477,465	109,597	0.9	2.8
Bengal	46,109,157	68,809	0.6	1.5
	3,06,406,463	12,170,439		

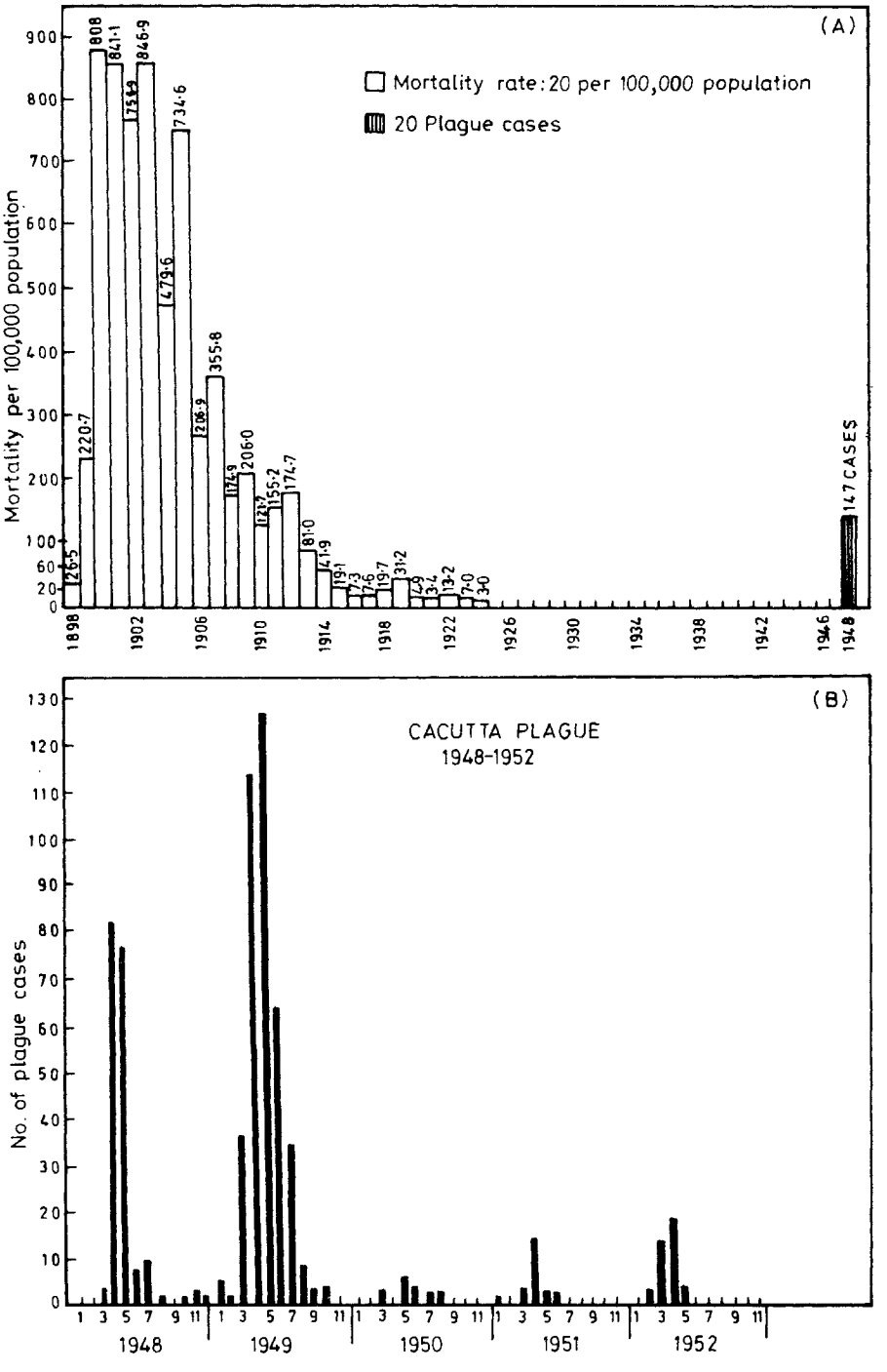

(A)
Mortality rate: 20 per 100,000 population
20 Plague cases
Mortality per 100,000 population
147 CASES
(B)
CACUTTA PLAGUE
1948-1952
No. of plague cases
1948
1949
1950
1951
1952

Figure 4 (A & B)

rats *(R. norvegicus)* which may be of sylvatic origin. It takes about 10 days for the epizootic to appear in the house rats and about another 10 days to involve the human beings (figure 3).

The peculiarity of plague epidemics, particularly in cities and towns is that after its introduction or reappearance it goes on for a few years before a time interval is reached, and usually bigger the city the longer is the range of continuity. In a big city like Calcutta where there is enormous rat population it takes several years for a large size epizootic to develop, which is maintained for several years due to almost continuous supply of susceptibles in sufficient number (thus raising the herd immunity) and to bring about cessation. It is also noticed that in the beginning small outbreaks occur which gain momentum within 3 or 4 years unless seriously interfered within its earlier phase of the first 2 years (as in case of the last Calcutta epidemic—figure 4a) and then a heavy epidemic occurs for some years followed by gradual fall through several years till it disappears for some time (figure 4b). The abrupt decline of epidemic wave as a premature cessation of transmission is considered as "incomplete" epidemic because it terminates before its herd immunity has reached the climax. This is what happened in the last Calcutta epidemic due to prompt and effective early action by the author. The world prevalence of plague at the time of Calcutta epidemic is shown in figure 5.

Morbidity, mortality and spread: Cases are generally severe in the early phase of the epidemic. With the progress of the epidemic cases become less and finally are of mild type. All age groups may get the disease except the infants (0–6 months old). Sex and occupation have little significance but in Calcutta men suffered more than the females. M : F ratio may be partly responsible but women are better covered than males. The case fatality in bubonic plague varies in different epidemics, the fatality rate varying between 60 and 90% in untreated cases. It varies with climate, facilities for treatment, overcrowding etc. In the overcrowded Hong Kong and among the Chinese it was 93%, among the Japanese 60%, among the Indians 77% but among the Europeans 18.3%.

It usually spreads by contiguity but it may also spread to distant places through infected rats or fleas being carried with commodities or in person's body or clothes and also from one country to another through maritime and aerial transport. In a city the first appearance of plague in a locality may be followed by a rise in small foci (as in grain stores, ware houses, etc.) before covering wider areas. It has predilection to dirty insanitary dwellings, more particularly in the ground floor attacking generally poorer class of the people in the rat-infested huts and bustees, granaries, store rooms, refuse dumps which harbour rats.

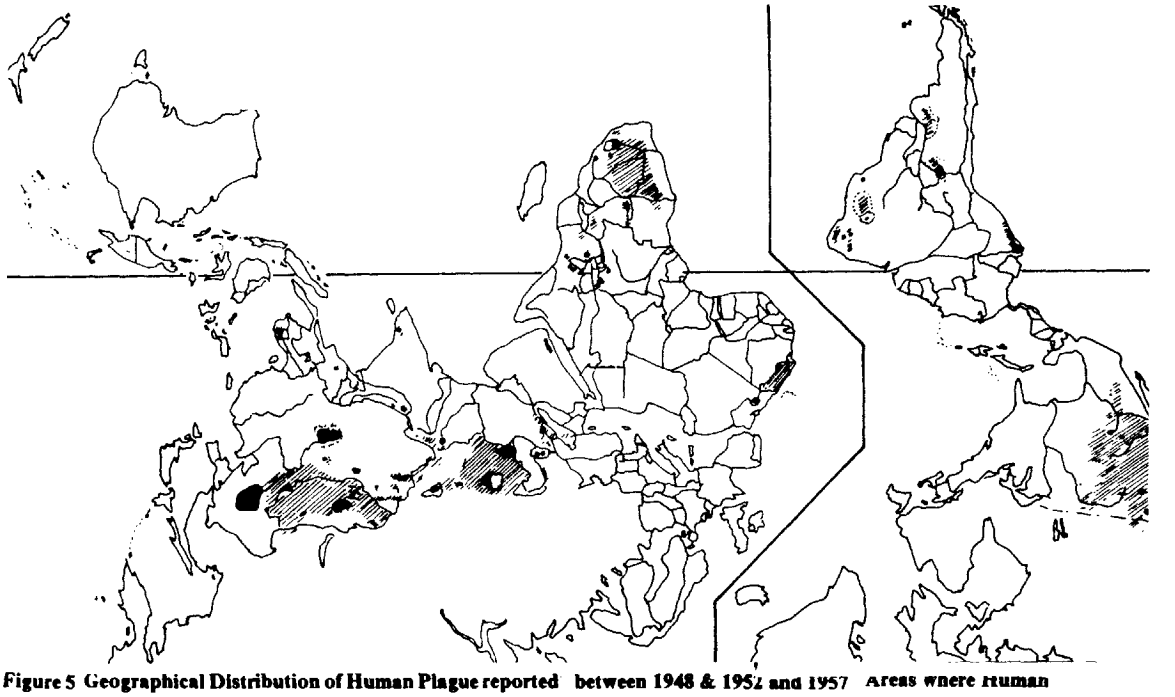


Figure 5 Geographical Distribution of Human Plague reported between 1948 & 1952 and 1957 Areas where Human Cases were Reported

Phase I

There were only three important discoveries during this phase namely, (i) confirmation of the role of rodents in the transmission of plague, (ii) discovery of plague bacillus in 1894, and (iii) the suggestion of transfer of infection through the maritime routes.

The relationship of rodents to plague actually dates back to the period earlier than 600 BC (vide *Bhagavata purana*). The Bible also mentions about 'mice' whose corresponding Hebrew word was "akbar" meaning rat. In all probabilities mice in the Philistine towns and ships were really rats (*Rattus alexandrianus*). It took a concrete shape in India by the 17th century when in 1616 the treasurer of Jahangir's court described how handling of dead rats led to plague infection in Punjab. A similar relationship was also established in China by Ling Chi (1736–1809). However, upto 1896 it was not known how the infection was actually being transferred from rats to man. It had to remain unsolved till the causative organism was discovered.

Another development during this period was the clinical distinction between bubonic, septicaemic and pneumonic plague. A definite idea about the pneumonic plague is, however, available in the book on '*Manchurian Outbreak* by Wu-Lien-Teh (1926).

Phase II

The actual scientific investigation about plague started when it visited Bombay and began to spread in all directions in India. Three Commissions were engaged in the study of the problems e.g., The German Plague Commission (1899), The Austrian Plague Commission (1895–1900) and the British Indian Plague Commission (1907–1912). The knowledge about the role of rats became clearer by the study of Bombay Plague (1896–1898) by the work of the pioneer French Epidemiologist, Dr Simond who between 1894 and 1898 working independently drew attention to the way plague was spreading in Bombay. By careful experimental device he proved that the infection was transmitted from rats to rat and human being through the bite of infected fleas, the ecto-parasite of rats. As is generally the fate of all new discoveries or theories, this new conception of bubonic plague as a flea-borne infection was opposed by many epidemiologists as well as the the Plague Commissions (British Indian and German). However, very soon support of Simond's observation began to pour in from the Japanese, French and Austrian workers. Gauthier and Raybaund (1903) successfully repeated Simond's experiment confirming his findings. Also Glen Liston (1906) in Bombay found evidence of the role of fleas by carrying out experiments with guineapigs which when placed inside the plague-infected house, not only imbibed infection and died but the fleas from their bodies also yielded plague organism. The rat-flea-man cycle was thus discovered as the missing link. This cycle is diagrammatically represented in figure 6a.

Mechanism of Transmission

Researches indicated that apart from mechanical transmission, which is possible during heavy epidemics, the usual mode of tansmission was through blocked fleas as discovered by Bacot and Martin (1914). They showed that infection was generally transmitted by fleas whose gizzard and proventriculus had been blocked by growth of plague bacillus. Under the circumstances the fleas being unable to suck blood into their stomach regurgitated the organisms by elastic recoil of their distended oesophagus and pharynx back into the wound made by their probocis together with masses of plague bacillus detached from the obstructing growth. The blockage phenomenon can be easily seen under microscope or even hand-lens (figure 6b).

The next stage was to identify various rodents involved in the transmission of plague infection namely: (i) wild rodents, (ii) peri-domestic rodents, and (iii) domestic rodents.

Some of the wild rodents are tarabagan, susliks, jerboa, gebrils, wood rats, ground squirrels, striped mice, tatera etc. and the peri-domestic rats are tatera indica, funumbulus palmarum, mus buduga, gunomys kok (field rats), while domestic rats are *Rattus rattus, Rattus norvegicus, Rattus rattus alexandrianus, Rattus frugivorus, B. bengalensis* etc. (figure 7).

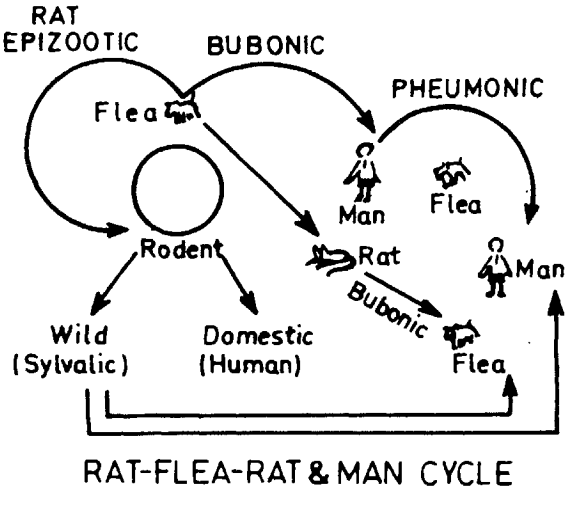


Figure 6 A Plague Cycle

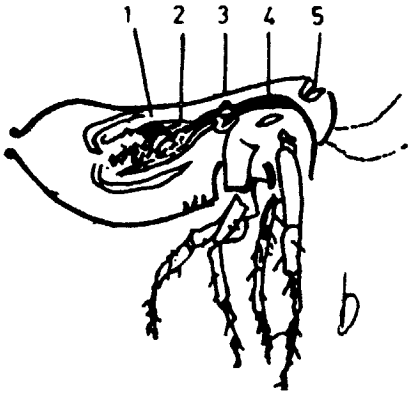


Figure 6 B Diagram of blocked rat flea (Bacot & Martin) **1,** Mases of *P. pestis.* & stomack; **3,** Proventricular value blocked; **4,** Oesophazus with masses of *P. pestis* **5,** Pharygeal pump.

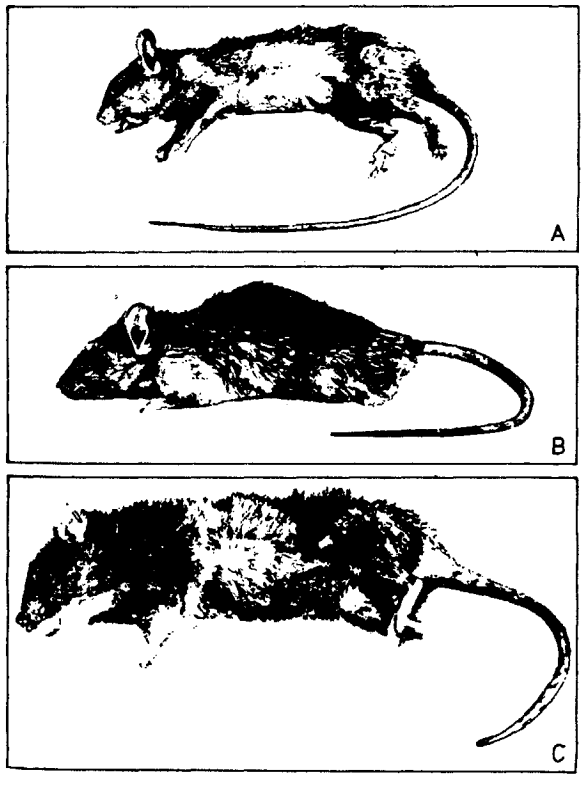


Figure 7 A-C, A *Rattus rattus rattus,* **B,** *Rattus rattus norvegicus* **C,** *Bandicota bengalensis Kok (Gunomys Kok Aüct.)*

The above is the nutshell of the position about the knowledge of plague till 1940 when the author took up the study. On taking over he found the position as follows:

There was hardly any method of treatment or clear-cut quick method of identification of plague bacillus, culture from both liquid and solid media being rough and auto-agglutinable. Also knowledge about specific chemical antigenic structure remained vague nor the antigen could be isolated in pure form. The bacillus was taken as normally rough ignoring the fact that roughness is only a dissociant form of bacteria in general and antigenically poorer than that of the smooth organism. So the use of this organism yielded but poor vaccine and also poor antiserum which was the only effective method of treatment before the discovery of sulpha drugs and streptomycin. The author therefore concluded that the fundamental defect lay in the culture media and was virtually a problem of nutrition of plague bacillus. He also noted that due to the lack of this knowledge, for growing the organism in the media used required a heavy inoculum and 4–6 hr lag period. What was happening is that the media containing half-digested protein of the meat was not immediately suitable for the growth and nutrition of the plague bacillus. The heavy inoculum resulted in the death of a portion of the bacterial mass and this provided the proper nutrition for their growth and elaboration of the enzyme for the digestion of the protein to convert it into amino-acid form needed for their growth and for which the lag period was necessary.

Discovery of a New medium

Having reached at this conclusion the primary need was to work out an appropriate and suitable nutritive medium which would provide the ready made nutritive substance to grow without any lag period. Thus his discovery of the protein-free casein hydrolysate medium in connection with the culture of diphtheria organism came very handy for the purpose by enrichment with a very small quantity of liver extract. In this medium growth starts with the smallest inoculum (even as low as 10 or 20 organisms) and a count made every half hours shows that the optimum growth of 193 million/ml was reached in 54 hr (table 2) and that this optimum growth could be obtained with hydrolysate of 72 hr digestion. The final preparation of the medium was as follows:— (Seal 1943, Seal & Mukherjee 1950).

Liquid Medium: Casein is hydrolysed by combined HCl and H_2SO_4 by digesting it for 72 hr. It is then neutralised by $Ba(OH)_2$, $8H_2O$ and filtered, and nitrogen content estimated by micro-Kjeldahl method. To the filtrate 0.2 mg of anhydrous Na_2HPO_4 and 0.067 mg of KH_2PO_4 are added per mg of N, followed by 0.04 mg of $CaCl_2.2H_2O$. The *p*H is adjusted between 7.4 and 7.6 with NaOH and the mixture heated

Table 2 *Viable counts/ml of growth of* Y. pestis *in casein hydrolysate broth medium in relation to the period of incubation*

Period of incubation (hr)	C H basal	C H with liver extract	Period of incubation (hr)	C H basal	C H with liver extract
(Seed)	556	420	18	28×10^2	266×10^3
½	—	595	24	155×10^3	286×10^4
1	858	645	30	85×10^4	25×10^6
2	615	1,005	36	77×10^5	65×10^6
3	750	not done	42	872×10^5	106×10^6
6	2,800	4,450	48	98×10^5	not done
9	4,400	not done	54	1075×10^5	193×10^6

CH, Casein hydrolysate

for 5 min. The precipitate thus formed is filtered off through paper and the filtrate diluted to contain 2.7 mg N/ml. Then $MgSO_4.7H_2O$ and $FeSO_4.7H_2O$ and liver extract are added in doses of 0.5, 0.0004 and 0.1 mg, respectively, per ml of the medium and the *p*H readjusted, if necessary. The medium is then distributed in flasks and tubes and sterilised in the autoclave at 110°C for 10 min.

The advantages of this medium are:

(i) Autoagglutination is completely removed;
(ii) Facilitates isolation of specific soluble proteins in pure form by precipitation with sodium or ammonium sulphate;
(iii) Facilitates production of improved quality of Haffkine plague vaccine and of antiplague horse serum for treatment of plague cases;
(iv) Permits study of the chemical antigenic structure of the organism;
(v) Solid medium in the form of casein hydrolysate agar gives smooth colonies and produces a smooth suspension for agglutination test and study of specific proteins and polysachharide of the plague and allied bacilli;
(vi) Antisera produced against different fractions of specific proteins helps in differentiating *Y. pestis* from *P. pseudotuberculosis* and other organisms;
(vii) Antigens isolated from supernatant of the growth of the organism in casein hydrolysate medium can be directly used as vaccine, and can be indefinitely maintained as a stock, to be used in any emergencies as well.

Solid medium for the culture of plague bacillus: Ordinary nutrient agar gives poor growth of plague bacillus. Sokhey (1939) found 5% rabbit blood agar most suitable for the purpose but the drawback is that vaccine prepared out of growth of plague organism will also contain some antigen of rabbit's blood which may give rise to adverse antigenic reaction apart from the large quantity of rabbit blood that would be necessary for the production of vaccine on a large scale. Casein hydrolysate agar (Seal 1950) was found to be equally as good as the 5% rabbit blood agar barring the probable undesirable reaction. Besides, plague culture on CH agar can be advantageously used for the preparation of potent antiplague vaccine as well as antisera and also for isolation of specific soluble antigen for serological work namely, agglutination, precipitation and complement-fixation work and estimation of specific antibody against plague infection in both man and rodents on a large scale by gel-precipitation test (to be described later).

Capsule, Envelope and Virulence

There was no unanimity of opinion about the presence of both capsule and envelope being present in the plague bacillus. The demonstration of both actually depended upon the efficiency of the culture medium as well as on the technique of staining. With the improvement of the culture medium the present author (Seal 1959) was able to demonstrate the existence of both in the virulent plague bacillus. By a new technique *Y. pestis* grown on 5% rabbit blood agar or CH agar at 37°C showed the largest amount of envelope substance and smallest amount in strains grown on A-D agar. The avirulent non-protective plague and pseudotuberculosis strains did not show any envelope (figure 8). The organism is suspended in few ml of distilled water and

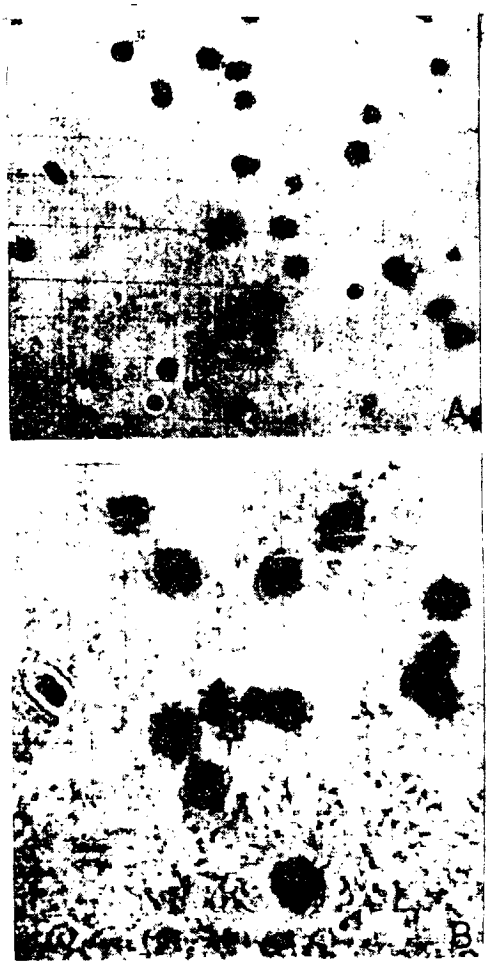


Figure 8 A & B. **A**, Showing the capsule and the core of the organism and an unstained hallow around; **B**, Showing the envelope and the core of organism differentially stained by a special technique

after 5 min a drop or two of formalin is added to this suspension and then stained with methylene blue by adding a few drops into the suspension. Under the high power microscope envelope is seen as a light-coloured membrane around the capsule stained a little deeply.

Types of Plague bacilli

Originally plague bacillus was placed under *Pasteurella* but lately it has been classified in the group called *Yersinia* after its discoverer. Bezsonova (1930) divided the strain into two types: (i) Glycerol-positive, and (ii) Glycerol-negative. The strains isolated in south-east Russia, Mongolia, Manchuria and Central Africa were found glycerine-positive, whereas strains isolated in India and south east Asian Islands, and Europe were glycerol-negative and nitrous acid positive as opposed to glycerol positive (fermenting glycerol) and nitrous acid negative. Devignat (1951, 1958) classified the strains into three types viz., *Var orientalis*, *Var mediaevalis* and *Var antiqua*. Thus, the author felt the needs for a thorough study of the chemical antigenic structure which can place all organisms into a stable and standard classification.

Virulence of Plague bacillus

Virulence and toxicity of plague bacillus are not synonymous but complementary. It not only means invasiveness but also combined with toxicity it brings about fatal termination of the infected man or animal. Virulence is determined by animal inoculation test. Only 5–10 virulent organisms are required to kill 50% of mice (Sokhey 1939). The newly isolated organisms are tested against this standard for virulence. Plague strains of low virulence have been detected by the author both in the course of an epidemic as well as during the interepidemic period among the rats. Virulence of such organisms can however be enhanced by passage in mice. These two observations taken together provided the clue to the mechanism of plague to rise in epizootic form from the pre-epizootic to the epizootic stage and then to human epidemic Accordingly, the author planned and carried out experimental epidemiology to be reported later.

Toxicity of different types of Plague vaccines

One of the objectionable features of the Haffkine plague vaccine was its toxicity. Once it was even thought that the response to immunity depended upon this reaction but the comparative tests of toxicity as well as of the protective quality of different vaccines carried out by the author (table 3) indicate that the vaccine prepared in casein hydrolysate broth was least toxic and yet gives better immunity.

From table 3 it is apparent that the growth in CH Agar medium is the least toxic and better protective than the growth in casein hydrolysate broth and Haffkine plague vaccine which was found to be the most toxic and comparatively least protective. So the advantages lie with the hydrolysate media. A comparative colony characteristics in different solid media are shown in figure 9 (Seal 1950).

The growth on A-D agar was not only poor but also appeared more rough than the serum or rabbit blood agar and formed unstable suspension in 0.85 salt sol. The growth on rabbit blood agar and C H agar appeared as smooth colonies and formed homogeneous and stable suspension in normal salt solution. Virulent *Y. pestis* also showed some dew drop like colonies on these two

media. The colonies of the avirulent non-protective plague and pseudotuberculosis strains were comparatively more opaque with occasional smooth colonies. The special advantage of rabbit blood agar is its capacity to maintain virulence and other antigenic structure in stab culture in Frigidaire with only one subculture annually. While the growth of virulent plague bacillus gives a uniform turbidity in casein hydrolysate broth, the avirulent plague strains give somewhat granular turbidity. The classical stalactitite growth or the pellicular growth leaving the media more or less clear, and deposit at the bottom which, according to the author is nothing but evidence of roughness and lack of proper nutrition (figure 10).

Reaction on Carbohydrates (sugars)

The author also brought about improvement in the carbohydrate fermentation reaction employing 19 sugars for 35 strains of both plague and pseudotuberculosis organisms (Seal 1951 a) The results are shown in table 4.

The sugars that distinguish pseudotuberculosis from the plague strains are rhamnose and iso-dulcitol producing acid with the former and not with the latter strains. *P. avisepicus* did not ferment maltose and rhamnose but fermented sachharose and sorbitol, thus differentiating it from both plague and pseudotuberculosis organisms. To distinguish pseudotuberculosis strains from one another the three sugars viz., raffinose, treahalose and dextrin were found useful, dextrin being fermented by PR/I, trehalose by PR/II, raffinose by PR/III and both raffinose and trehalose by PR/IV. (PR = *Pseudotuberculosis rodentium*).

Table 3 *Toxicity and mouse protective doses of three different plague vaccines*

Types of vaccine	Mouse-protective dose	Toxic dose
Haffkine plague vaccine	0.006 ml	0.2 ml
Casein hydrolysate broth (1000 million organisms from 3 days' growth)	0.0053 ml	0.8 ml
C H agar vaccine (1000 million organisms per ml)	0.0048 ml	1.5 ml

Table 4 *Differential sugar fermentation reaction by different strains*

Strains	Sugars								
	Maltose	Rhamnose	Raffinose	Sachharose	Trehalose	Insulin	Dextrin	Sorbitol	Iso-dulcitol
Plague strains	A	0	0	0	A	0	A	0	0
PR/I	A	A	0	0	0	0	A	0	—
PR/II	A	A	0	0	A	0	0	0	—
PR/III	A	A	A	0	0	0	—	0	A
PR/IV	A	A	A	0	A	0	—	0	A
P.avisepticus	0	0	0	A	0	A	0	A	—

0, not fermented; *A*, fermented; —, not done

Proposed subgroup of *Y. pestis*

Proposed subgroup	Test with glycerol	Production of nitrous acid	Remarks
***Y. pestis* var orientalis**	—	+	Oceanic race (Berlin & Borsekov 1938)
***Y. pestis* var antique**	+	+	Found in Central and North Asia and in Central Africa
***Y. pestis* var mediavalis**	+	+	Found in South-East Russia

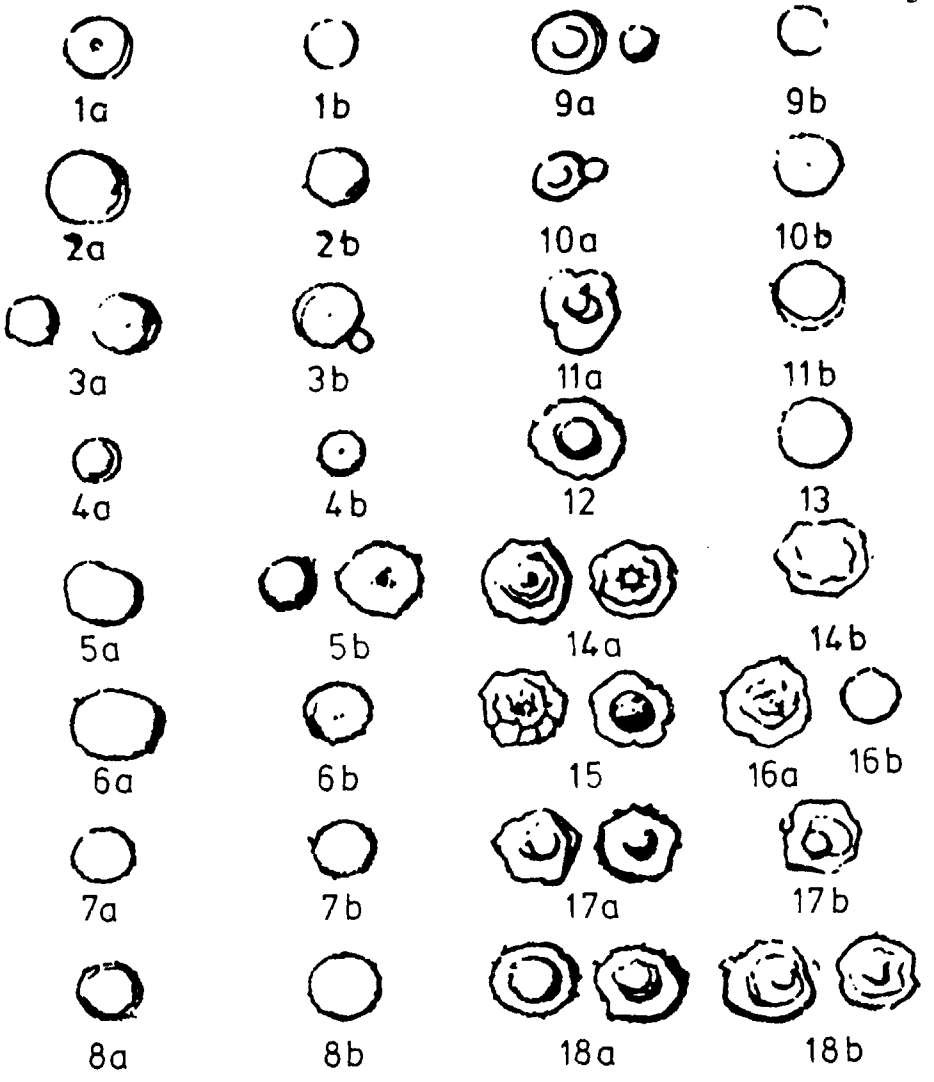


Figure 9 Colonial characters of the various plague and psoudotuberculosis strains on 10% sorum and 51 rabbit-blood agar media.

1. (a)	Virulent	*P. pestis*	14/L	on serum	agar;	(b)	the same	on rabbit-blood	agar.
2. (a)	"	"	27/L	"	"	(b)	"	"	"
3. (a)	"	"	149/B	"	"	(b)	"	"	"
4. (a)	"	"	308/B	"	"	(b)	"	"	"
5. (a)	"	(old)	54/H	"	"	(b)	"	"	"
6. (a)	"	"	337/L	"	"	(b)	"	"	"
7. (a)	"	"	139/L	"	"	(b)	"	"	"
8. (a)	Av protective	*P. Pestis*	53 Har	"	"	(b)	"	"	"
9. (a)	"	"	EV	"	"	(b)	"	"	"
10. (a)	"	"	Tjs	"	"	(b)	"	"	"
11. (a)	Av non protective	"	120/5 Har	"	"	(b)	"	"	"
12. (a)	"	"	I/av	"	"	13.	TJR on	serum agar	"
14.15, 17 & 18	2nd 16 non-protect	av. p pestis						Pseudotuberculosa Strains PRI and PR (II)	

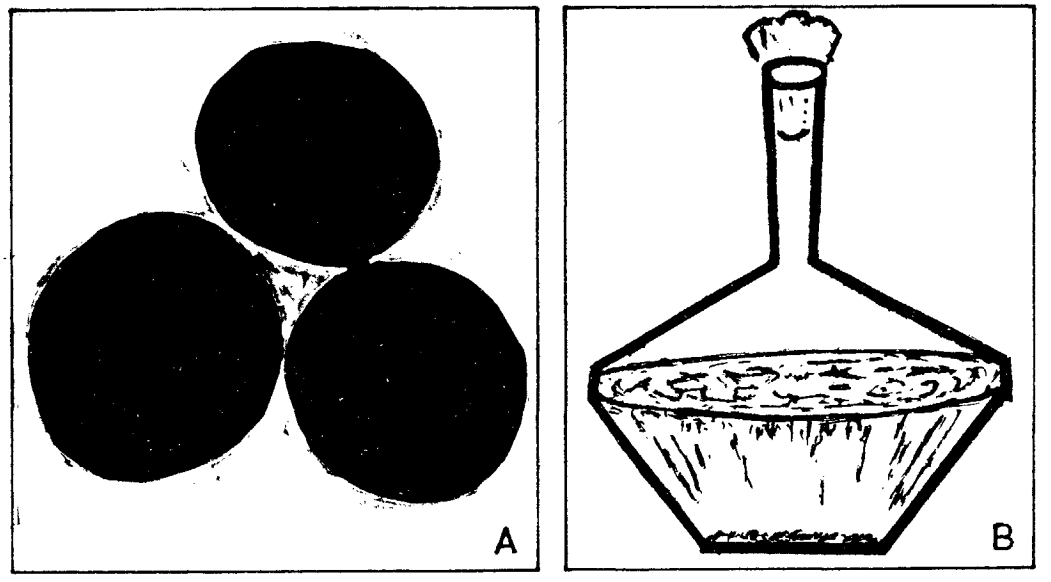


Figure 10 A & B, A, Smooth Colonies of Virulent plague strains, **B,** Culture of plague bacillus in Haffkine Flash

Identification of Chemical Antigenic Structure of Plague and Other Strains

As mentioned earlier the plague workers were facing difficulties in the identification of the organism due to autoagglutination and in isolating the specific antigen, as the media contained non-specific proteins coming in the way for purification of the antigen. Both the difficulties were overcome by the discovery of the protein-free casein hydrolysate medium. A third innovation was introduced in the agglutination reaction by using live plague culture instead of killed or chemically treated suspension. The problem actually lay in the differentiation between plague and pseudotuberculosis strains, both of them being the carrier and infecting organism for the rats. Another difficulty faced by them is the differentiation between virulent and avirulent protective and non-protective strains, all the three having been found by the author to play some role in the epidemiology of plague. Burrows and Bacon (1956) by a special technique discovered two additional antigens called V and W which can differentiate virulent from the avirulent plague strains being present in the former and absent in the latter. In a subsequent study Burrows (1959) discovered two additional determinants of virulence namely, P (pigment-positive) and Pu (Purine producing). Accordingly the serologic antigenic structure of plague and pseudotuberculosis strains were defined as follows (table 5).

Chemical Antigenic Structure of Plague and Allied Organisms

Various workers from time to time attempted to isolate specific antigens or antigens in pure form (Schutze 1932, Sokhey & Maurice 1935, Sokhey 1939, and Baker et al. 1947) all faced the difficulty of freeing it from the non-specific part mixed up with it although they noted that it was a protein or a nucleoprotein. Baker's work also suffered from the defect in chosing nutrient agar which was not fully nutritive to plague organism. Author's casein hydrolysate medium seemed to be the ideal in this respect (Seal 1951b).

Technique of Isolation in Brief

From *casein hydrolysate broth:* Casein hydrolysate broth was inoculated with virulent plague strain grown on either 5% rabbit blood agar, casein hydrolysate broth or casein hydrolysate agar for 48 hr at 37°C, and incubated at 37°C for 2-3 weeks and filtered. The clear filtrate is then precipitated at different saturation of Na_2SO_4 to yield P⅓, P½; P½-⅓ and P_1-½ fractions.

(b) From casein hydrolysate agar: 72-hr culture of *Y. pestis* virulent was inoculated into the CH agar in Roux bottles and incubated at 37°C for 48 to 72 hr and

Table 5 *Antigenic structure of plague bacilli as defined by Burrows (1959)*

Strains	Antigens	Antigenic character
Y. Pestis virulent	Fl+, VW+, P+, Pu+	Common rough somatic antigen
Y. pestis avirulent protective	Fl+, VW−, P+ or −Pu+	--- do ---
Y. pestis avirulent non-protective	Fl−, VW−, P+ or−, Pu−	--- do ---
P. pseudotuberculosis (fresh)	Fl−, VW+, P+, Pu+	Flageller, smooth somatic (type and group specific)
P. pseduotuberculosis (laboratory)	Fl−, VW−, P+, Pu+	Common rough somatic

Fl antigen of Baker et al. is the same as antigen A of Seal (1943, 1950, 1951 d, 1953, 1954)

washed with distilled water and collected in a flask in strength of an optimum of 600 million organisms per ml (by matching with opacity tubes) and incubated for 12–14 days by which period all suspended organisms would be found dissolved (culture negative). The material is then filtered and the filtrate precipitated by Na_2SO_4 in the same saturation of Na_2SO_4 to yield the same four fractions as in the case of casein hydrolysate broth.

Chemically tested, all the protein fractions were sulphur phospho-proteins. Tests for tryptophane and tryosine showed that the P½ fraction obtained from the protective strains grown in CH broth contains both these amino-acids and those from non-protective plague and pseudotuberculosis strains showed little or none, whereas those from the water-soluble extracts did not contain any of them. Since tryptophane was virtually absent in the CH broth its presence in the fractions apparently indicate that the plague bacillus could synthesize this amino-acid during its growth in CH broth.

Isolation of Active Polysaccharide from Plague bacillus

In the study of chemical antigenic structure it was of interest to know if any polysaccharide moiety was involved in the specific soluble protein antigen, as we had in case of *V. cholerae*. Attempts were therefore made to isolate this substance from three sources e.g., (i) supernatant of antiplague vaccine; prepared on casein hydrolysate broth; (ii) the bacterial debries of the same vaccine; (iii) specific soluble protein of plague bacillus.

A polysachharide corresponding to *"arabinose"* yielding osazone with melting point of 166–168°C was isolated more or less from each of the above. It was absent in the non-protective plague and pseudotuberculosis strains. Therefore, the specific soluble protein of plague bacillus is a poly-saccharide-protein complex, i.e. a nucleo-protein derivative (Seal 1951 c).

The results of agglutination and cross-agglutination titres of the anti-protein sera against various plague and allied organisms (table 6) show $L_{Auto}P⅓$* is highly specific plague bacillus so far as the serological reactions are concerned. This fraction is not present in the precipitate of pseudotuberculosis organism or in any of its fractions. Thus $L_{Auto}P⅓$* fraction may be suitably utilised for differentiating protective from non-protective or pseudotuberculosis strains but not the virulent from the avirulent protective plague strains. On the other hand, the fraction P½–⅓* of virulent plague strain reacting with the plague and pseudotuberculosis strains may be the antigen that maintain the antigenic link between the virulent plague and pseudo-tuberculosis organisms. It is also related to 'O' anitgen of the virulent plague bacillus as seen in table 7.

Thus the relationship of plague to the pseudotuberculosis strains is through the somatic antigen represented by the P½–⅓ fraction of broth filtrate of virulent plague bacillus.

Accordingly, the fraction P⅓ of virulent plague bacillus has been designated as Antigen A and P½-⅓ as Antigen B, the latter serving as the link between the plague and pseudotuberculosis organisms.

The other advantages of the specific soluble proteins are: precipitation test, ring precipitation test and complement-fixation test, all of which can specifically identify the plague organism. While the gel-precipitation test is to test immunity status of rat population on a mass sole in the field, and lastly for standardisation of anti-plague sera by quantitative flocculation test. A brief reference to these tests may only be made.

Procedures for Precipitation Reactions

For direct and cross-precipitation test the specific protein, antigen A, diluted to contain 0.1 mg/ml

*A_{Auto}: *L*, Lot; *Auto*, Autolysate

Table 6 *Agglutination and crossagglutination titres of the anti-protein sera against various plague and allied organisms (min. dil.1:25)*

Anti-protein sera	Organism (suspensions)					
	337/L	53H/av	120H/av	TRU	PR/I	PR/IV
L/auto $P^1/3$	400	200	0	0	0	0
$L_5P^1/3$	200	200	25	0	—	—
$L_5P^1/2\text{-}^1/3$	0	0	50	25	25	25
$L_5P^1/2$	100	100	25	25	25	25
53H/av$P^1/3$	100	100	25(f)	0	0	0
53H/av$P^1/2\text{-}^1/3$	0	0	100	25	25	25
120H/av$P^1/3$	0	0	100	100	25	25
120H/av$P^1/2\text{-}^1/3$	0	0	100	±	25	50
$TRUP^1/3$	0	0	0	50	25	25
$PR/IP^1/3$	0	0	25	50	50	25
$PR/IP^1/2\text{-}^1/3$	0	0	25	50	50	50
$PR/IVP^{1/3}$	0	0	25	±	25	50
$PR/IVP^1/2\text{-}^1/3$	0	0	50	±	50	50

337/L, vir, plague; 53H/av, av. protect. plague; 120H/av, av non-prot. plaugue PR/I and PR/IV, pseudotub. organism
$P^1/3$, 33% saturation of Na_2SO_4. $P^1/2$, 50% saturation of Na_2SO_4

Table 7 *Result of agglutination test between the virulent plague strain 337/L boiled and the pseudotuberculosis strains*

Antiserum against 337/L 'O' (boiled)	Pseudotuberculosis strains			
	1104/B	3572	3570	1093/25
Result	+ +	+ +	+ +	+ ±

(1:10000) is distributed in Felix tubes in 0.5 ml amounts. The tubes are lightly shaken and incubated at 37°C for 2 hr. A preliminary reading is taken and the tubes are placed in the refrigerator for the final reading next morning. The highest dilution showing the visible precipitation is taken to be the titre of the serum.

Ring Precipitation Test

For quicker diagnosis, ring precipitation test may also be done in the following manner: Antigen A 1/1000 dilution is taken in Dryer's tube and the patient's undiluted serum or 1:2 to 1:10 dilution is added on the top of the antigen by means of a capillary pipette. A precipitating ring develops in 1/2 hr. at room temperature. Even the filtrate of casein hydrolysate broth inoculated with unknown organism or the water extractable filtrate of distilled water suspension of the unknown organism may be used for both ring and tube precipitation tests.

The precipitation titres of various antisera as obtained against $L_2P^1/2$ fraction (antigen A) are given in table 8.

The result described above thus correspond exactly with those of agglutination test.

Antigenic Classification of Plague Strains

On the basis of the above analysis the plague strains were classified into three goups namely

(i) those containing both A and B antigens e.g. all virulent and relatively avirulent strains;
(ii) those containing a small amount of residual antigen A and full antigen B e.g. relatively avirulent TJS strain;
(iii) Those containing only antigen B, e.g. avirulent non-protective plague organism like TRU and all pseudotuberculosis strains.

Spectrographic Analysis of the Protein Fractions

A difference between virulent protective and avirulent protective plague strains could be made only by spectrographic analysis of the fractions. The absorption curve obtained by plotting the extinction coefficient against wave lengths the absorption range of all the protein solutions (made by dissolving in N/80 NaOH to make 0.04 per cent clear solution) lay near the ultra-violet region between 230 and 430 m (figure 11). In the case of protein fraction L_3 $P^1/3$ and $L_{Auto}P^1/3$

Table 8 *Precipitin titres of various antisera against $L_2P^1/2$ protein fraction*

antisera	Live antigen used for immunisation	Titre of $L_2P^1/2$ fraction
54/H rabbit	virulent *Y. pestis*	2,000,000
H/23 of 2.11.41	virulent *Y. pestis*, vaccine filtrate and relatively avirulent E.V. strain	1,000,000
H/27 of 2.11.41	virulent *Y. pestis*, vaccine filtrate and relatively avirulent strain, Tjs	1,000,000
H/45 of 2.11.41	Relatively avirulent Tjs only	1,000,000
H/44 of 2.11.41	Relatively avirulent Tjs only	50,000
E.V. Rabbit	Relatively avirulent *Y. pestis* E.V. Strain	500,000
Tjs Rabbit	Avirulent protective *Y. pestis*	500,000
Tjs Rabbit	Avirulent *Y. pestis* Rough	200,000
TRU	Avirulent non-protective *Y. pestis*	Cloudy only
PR/I Rabbit	Pseudotuberculosis strain	-- do --
PR/II Rabbit	--- do ---	-- do --

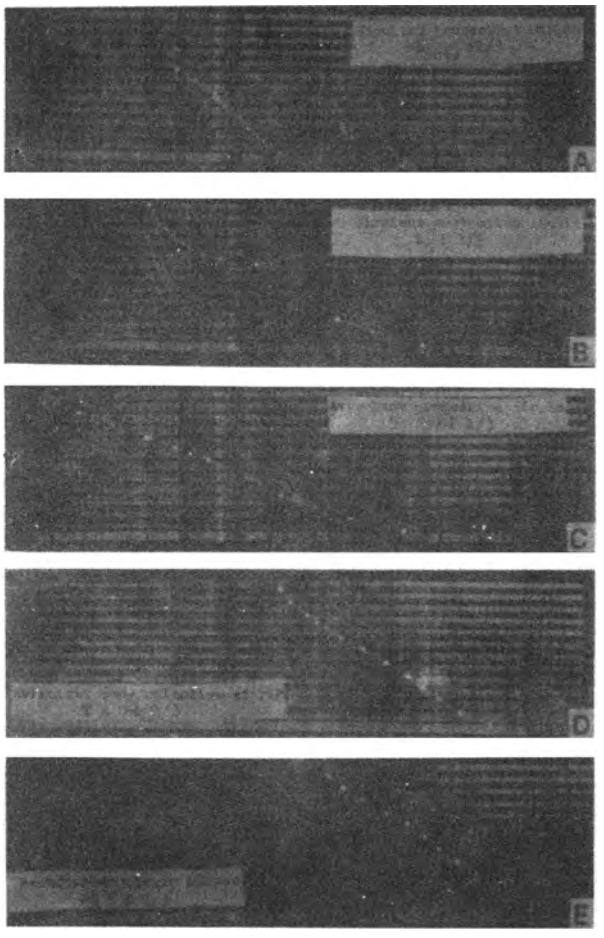


Figure 11 Spectro-photographs of protein fractions of plague and pseudo-tuberculosis strains. **A,** Virulent protective Plague Strain L_{auto} P1/3; **B,** Virulent protective Plague strain L_5 P1/3; **C,** Virulent protective Plague strain 53 Hav P1/3; **D,** avirlent non-protective Plague strain TRU P1/3; and **E** Pseudotuberculosis strain PR/1 P1/3

fractions isolated from virulent *Y. pestis* had a moderate bend in the absorption curve between 268 and 305 m which was not seen with the other preparations. This might be taken as evidence of difference between virulent and avirulent plague strains (Seal 1960a).

Complement-fixation Test

The author (1953) utilised specific soluble plague proteins for complement fixation test. No cross-reaction was noted with antisera against either the whole of avirulent non-protective strains or of the pseudotuberculosis strains.

Technique of the test: Antigen A (P½ fraction) is diluted to 1:4000 to 1:5000 in 1% NaCl solution. The test sera are inactivated by heating in a water bath at 54°C for 10 minutes. Fresh complement is obtained by bleeding guineapigs each day and is used after titration as in the usual case of Wasserman Test. *Titration:* Tubes are placed in a series and 0.25 ml of various dilutions (1:4 to 1:200) of inactivated sera is added to them, followed by 0.25 ml of 1½ to 2 MHD of complement. The contents are then mixed well by shaking and the tubes incubated at 0 ± 3°C for ½hour. Sensitized cells are then added in 0.25 ml amounts and the tubes are shaken and incubated for half an hour at 37°C. A preliminary reading is taken and the tubes are left in the refrigerator overnight and the final reading is taken next morning. The positive results are recorded 1+ to 4+ according to the haemolysed cells settled at the bottom. Negative is indicated by complete haemolysis. The results of complement fixation tests with protein fractions against the various antiplague and anti-pseudotuberculosis sera are given in table 9.

In this test also $L_{Auto}P_{1/3}$ proved to be the most specific followed by other protein fractions which were somewhat broader such as L_5 P⅓ which reacted with the PR/I pseudotuberculosis antiserum. Some workers however opine that this test may be advantageously utilised for the diagnosis of rodent plague in the field especially when the isolation at autopsy is rendered difficult by contamination or decomposition. This is no longer needed since the discovery of gel-precipitation test by the author (1972a), a much simpler method than the complicated complement-fixation test for the diagnosis of wild rodent plague.

Gel-precipitation Test

Preparation of the the gel plate: A gel prepared with 1 per cent bacto-agar and 0.1 mg/ml of the antigen A (e.g., $L_{Auto}P_{1/3}$, steamed at boiling temperature of water is mixed with 100 units of penicillin-cum-streptomycin per ml after cooling and poured into petri dish as a thin layer and allowed to solidify. About 16 to 20 holes are

Table 9 *Complement-fixation tests of 139/L (vir. plague), 53H/av (av. protective plague) TRU (av. non-protective plague), and PR/I (anti-pseudotub.) sera with various protein fractions at dilution 1:5000*

Antigens	No.	139/L		53H/av		TRU		PR/I	
		1:25	1:50	1:25	1:50	1:25	1:50	1:25	1:50
$L_5P^1/_3$	A_1	4+	4+	+±	1+	±	0	2+	1+
$L/_{auto}P^1/_2$	A_2	4+	3+	+±	1+	±	0	0	0
53H/avP⅓	A_3	4+	4+	4+	3+	±	0	0	0
120H/avP⅓	B_1	2+	1+	1+	0	1+	0	0	0
TRU P½	B_2	±	0	±	0	2+	1+	1+	±
PR/I P½	B_3	1+	0	1+	0	±	0	4+	3+
PR/IV P½	B_4	0	0	0	0	0	0	0	0

cut into the gel. *The test:* Sera collected from the rats and preserved in the refrigerator are separately placed into holes of the plate by using capillary pipettes for each of the dilutions of 1:20 and 1:40. In the central hole a known antiplague serum is placed as positive control and one or two negative controls such as cholera or typhoid antiserum. The Petri dish is then incubated at 37°C for 24 to 48 hours (figure 12). The positive test is indicated by a clear opaque ring round the well. It is positively useful in surveillance operation in plague control work. Comparative values of the results of immunity test against plague infection in Calcutta and Murarai (West Bengal) in 1971 are shown in table 10.

The above data shows that the immunity test with gel-precipitation test is simpler, quicker and also more reliable.

Serological Methods for Diagnosis of Plague

The various methods for sero-diagnosis of plague are agglutination, haemagglutination, precipitation, complement-fixation, and flocculation. The methods of precipitarion, complement-fixation and flocculation tests have already been discussed.

Agglutination

Although various workers tried to solve this problem of agglutination, it remained unsolved till the author became actively engaged in the work. With the improvement of the growth medium auto-agglutination

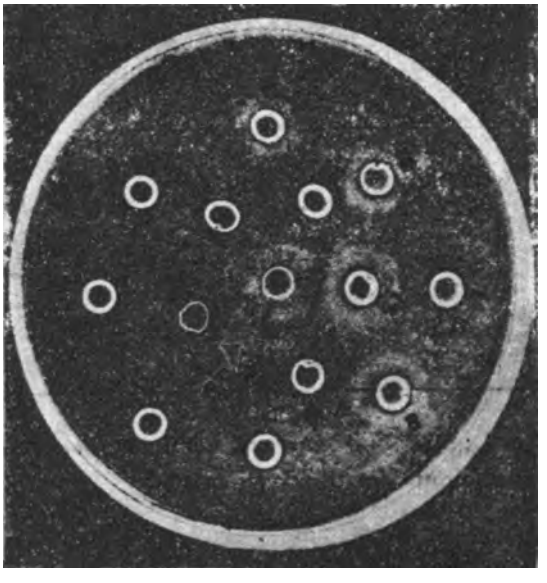


Figure 12 Gel-precipitation tests: Out of fourteen sera tested, sera Nos 5, 6, 8, 13, and 14, are positive

was completely removed. He made detailed study with antisera raised against virulent *Y. pestis*, *Y. pestis* boiled, for ½hr, *Y. pseudotuberculosis*, water-soluble protein extract of *Y. pestis* and with mutually absorbed sera (table 11). He showed that not only the difference between plague and pseudotuberculosis could be distinguished, the inter-strain differences among the plague strains could also be made (Seal 1952).

Antigen suspension: In this respect following the author's previous experience with vibrio agglutination (Linton & Seal 1935) by using live culture for

Table 10 *Comparative values of immunity test in rats against plague infection in Calcutta and Murarai (West Bengal) in 1971*

Place	Methods used	Percentage of rats found resistant		Total
		B.bengal-ensis	*B.rattus*	
Calcutta	Biological test	19.3	37.5	20.9
	Sero-agglutination test	32.7	42.1	34.0
	Complement-fixation test	28.3	36.4	29.6
	Gel- precipitation test	30.0	42.1	31.9
Murari	Biological test	19.3	24.8	22.0
	Complement-fixation test	33.3	25.0	23.0
	Gel-precipitation test	23.5	27.5	24.4

Table 11 *Serological relationship between* Y. pestis *and* P. pseudatuberculosis

Antisera produced against	*Y. pestis* vir. avir. Avirulent		*P. pseudo-tuber-culosis*
	protective	non-protective	
Virulent *Y. pestis*	+	+	+
Virulent *Y. pestis* absorbed with *P. pseudotuberculosis*	+	0	0
Y. pestis boiled for 30 min	0	+	+
Y. pseudotuberculosis	0	+	+
Water-extractable protein of *Y. pestis* (virulent)	+	0	0
Y. pseudotuberculosis absorbed with *Y. pestis*	0	0	+

preparing the suspension the same principle was applied in case of plague and its allied organism. By using the 24–48 hr growth on 5% rabbit blood agar or CH agar a smooth suspension of the plague organism could be easily obtained and so with pseudotuberculosis and other organisms. Only in case of non-protective avirulent plague strains an additional precaution of filtering the suspension through a pellet of sterile cotton wool was undertaken. For comparative study the various other types of suspensions used were: (i) heat-killed organism with or without preservative, (ii) boiled organism, (iii) alcohol-treated organism and (iv) specific soluble proteins (Seal 1951a).

Technique of agglutination: To various dilutions of antiserum placed in uniform bore Felix tubes equal amount of suspension is added and each tube gently shaken. The mixture is then incubated at 37°C overnight and the reading is taken next morning (a preliminary reading after 2 hours of incubation may also be recorded). The agglutination reaction was of two types viz., (i) floccular or woolly and (ii) granular (figure 13). The former is related to the surface or protective antigen and gives comparatively low titre agglutination, and the latter is related to the somatic antigen which generally gives high titre agglutination. The results of direct and series of cross-agglutination with absorbed and unabsorbed sera (Seal 1951a) gives the serologic relationship between different plague and pseudotuberculosis organisms as shown in table 11.

In the diagnosis of suspected case of plague or for retrospective diagnosis of human plague a slide agglutination test may help in quick diagnosis but the tube agglutination described above is the best. Using only No. 1 or 2 of the sera or only No. 5 reliable identification of plague organism can be made.

In the diagnosis of suspected case of plague or for retrospective diagnosis of human plague a slide agglutination test may help in quick diagnosis but the tube agglutination described above is the best. Using only No. 1 or 2 of the sera or only No. 5 reliable identification of plague organism can be made.

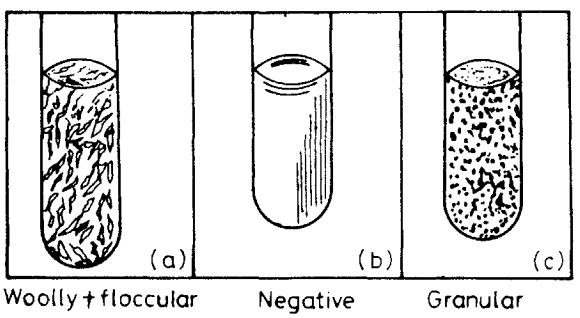


Figure 13

Out of 26 bacteriologically positive cases the sera collected between 26 and 130 days after cure 23 showed positive serological reaction and only 3 doubtful reaction while out of 56 suspected cases sera of 5 cases collected between 24 and 134 days after cure showed positive result. As a control, out of 55 non-plague cases 2 were found positive but these two cases gave history of receiving antiplague inoculation. Also by using this agglutination test the various pseudotueberculosis strains were differentiated.

Steps Towards Eradication of the Disease

In spite of the knowledge gained about the organism and its specific antigenic structure the main part of the work that still remained unsolved is the knowledge of how the epidemics arise, its periodicity maintained and what happens during the inter-epidemic period. Without this knowledge the question of effective control and final eradication could not be achieved. However, opportunities presented themselves by the reappearance of plague in Calcutta in 1948, the venue of the author's new assignment in the Epidemiology Department of the All India Institute of Hygiene and Public Health, Calcutta.

The last recorded case in Calcutta was in the year 1925. It reappeared in 1948 from the same area as in 1895. After the local authorities were convinced that it was an epidemic of plague the author's first task was to find out whether the source of infection was in the city itself or imported from outside. After a preliminary investigation carried out in collaboration with Professor Lal (Lal & Seal 1948) the author rejected the idea of importation of infection from outside. So the important epidemiological knot was the question of origin of infection and how it surfaced after 23 years. There was however one instance of positive isolation of plague bacillus in 1936 from a rat by a research student (Rao 1936) who wanted to study the actual situation underground. The present study also gave us the clue to the actual mode of spread of infection to human beings. The first human case was detected in Ward 8, the place where the first case of plague appeared in 1895. In the present outbreak dead rats were found in the house of the first case, which the inmates had to handle. Within a few days dead rats were picked up from several other wards of the city and interestingly rats in Ward 8 virtually disappeared. These two situations could be connected to find out the mode of local spread covering half a dozen wards within the span of a week.

Experimental study on Rat Movement

To further confirm the characteristics of rats, experimental studies were conducted on their short and

long range movements. Normally the range was found to be not exceeding 50 yards but when they were captured, marked and released 5 to 10 miles away from the place of capture they were found to retrace their paths towards the original place of capture figure 14 Seal & Bhattacharjee 1961, b).

Two other experiments were organised and carried out to obtain the final clue to the seasonal outbreak, and to the maintenance of carriers of infection for long periods among the rats as well as to study the mechanism by which an epizootic is caused followed by human epidemic.

Results of experimental work on the bionomics of fleas grown in the Laboratory: (Seal & Bhattacharjee 1961a).

The experiments (figure 15) show that while reproduction goes on all the year round in *X. cheopis*, the vector of plague infection has two waves of growth, one in the winter months immediately preceding and covering the epidemic wave of plague, and the other a smaller one during the rainy season, whereas *X. astia* has only one main wave of reproduction in the rainy season extending to autumn. During this reproductive period *X. cheopis* females increase greatly in proportion than the males but the condition is reversed in case of *X. astia*. These findings have been fairly corroborated in the field as well. The propensity of biting is however comparatively poorer in *X. cheopis* than in *X. astia* but it increases during the winter and spring.

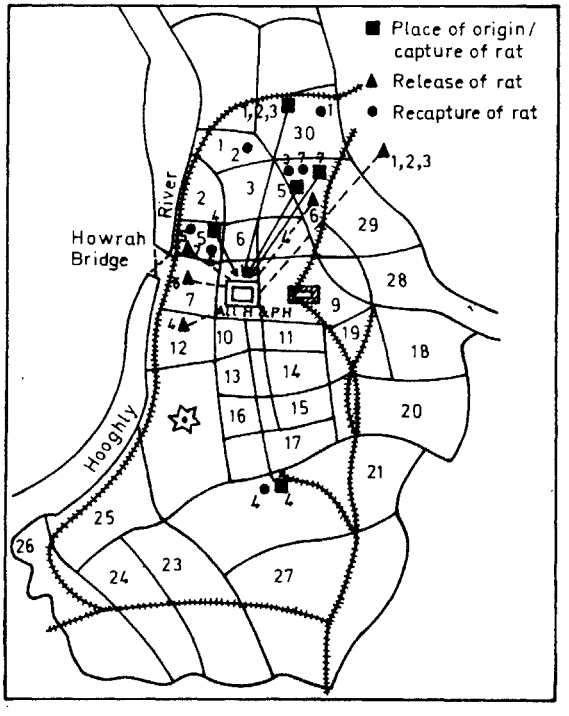


Figure 14 Map of Calcutta

As regards behaviour of rodents in the two wards (Ward 8, infected and Ward 10, uninfected) in Calcutta Ward 8 was found to be highly infected showing higher cheopis index and total flea population than in Ward 10. One of the reasons was the character of the housing conditions: The presence of bustees, godowns and huts in Ward 8 facilitated rat harbourage and infestations with house rats (*R. rattus)* more than in Ward 10. There were also large number of rat holes. On examination of these holes free fleas were found in 7.4% of the holes in April and in 18.5% in May, 21.0% in June and 0.0 in July and August. The specific flea indices were higher in Ward 8 than in Ward 10.

The result of resistance test also showed that all species of rats in Ward 10 were susceptible as against 60 per cent mortality in *R. rattus* examined towards the tail-end of the epidemic (1953–54) as shown in table 12.

The rodent densities also differed between the two Wards as will be seen in table 13. The difference is obvious.

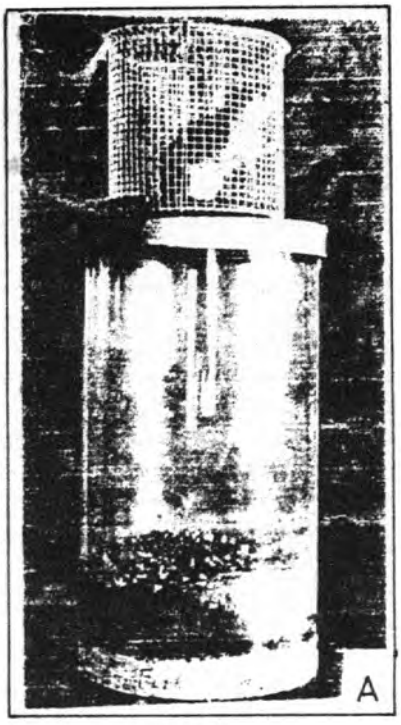
The above findings explain the existence of plague and its seasonal outbreak in one ward compared with another without plague.

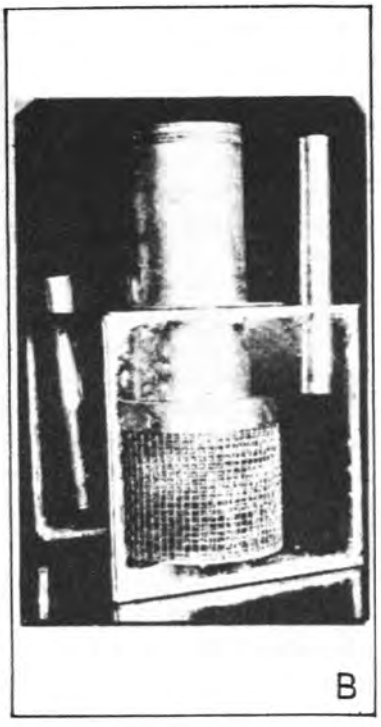
Continuous Natural Transmission Experiment

A continuous natural transmission experiment was carried out for 4 years through fleas among the two commonly available species of rats in Calcutta namely, *R. rattus* (domestic) and *B. bengalensis* (peridomestic). It was organised on the basis of experimental epidemiology and from the results obtained the following hypothesies were drawn regarding the mechanism of prepetuation of plague infection among the rodents bridging over the short and long intervals between epidemics (Seal 1961).

Table 12 *Resistance of rats caught in Wards 8 and 10 (1953–54); Test dose-5000* Y. pestis

Type of rat	Ward 8		Ward 10	
	No. tested	No. died	No. tested	No. died
R. rattus	30	18 (60)	22	22 (100.0)
R. norvegicus	28	26 (92.8)	18	18 (100.0)
B. bengalensis	50	49 (98.0)	55	55 (100.0)
Total	108	93 (86.0)	95	95 (100.0)





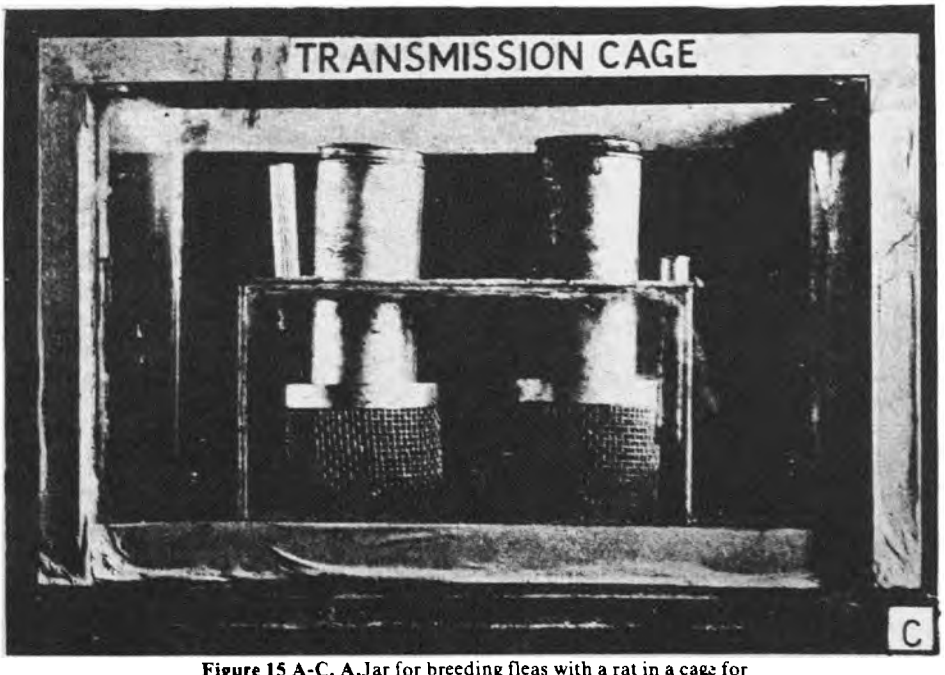


Figure 15 A-C, A, Jar for breeding fleas with a rat in a cage for feeding fleas; **B,** Single cage for trasmission experiment; **C,** Trasmission Chamber With double Cage

Table 13 *Rodent densities in Wards 8 and 10 by species and season (1952–53)*

Season	Rodent densities in Ward 8 (endemic)				Rodent densities in Ward 10 (non-endemic)			
	Total	Rr	Rn	Bb	Total	Rr	Rn	Bb
Jan-March	22.6	7.1	1.4	13.3	23.2	8.6	0.7	13.9
Apr-June	42.5	18.1	6.1	10.2	10.2	0.0	3.4	6.8
July-Sept	21.8	4.1	5.3	10.9	10.9	2.7	2.1	6.1
Oct-Dec	30.7	5.4	2.7	32.1	12.1	2.6	2.3	27.2
Whole year	29.8	8.5	3.9	22.5	12.5	3.8	11.9	16.8
Rats caught	498	148	65	291	445	75	38	332

Rr, *R. rattus;* Rn, *Rattus norvegicus;* Bb, *B. bengalensis*

(i) Apart from the clinical cases of plague among the rodent population infection can be maintained for prolonged periods in an inapparent or subclinical form in any of the partially immune rodents under investigation.

(ii) Depending on the environmental and other ancillary conditions plague infection lying in the dormant state (usually in the spleen) rises as a relapse with bacteraemic condition facilitating transfer of infection through the ectoparasite, gradually leading to epizootic and mortality among rats. The infected fleas thus released then serve as the vector of plague infection in man.

(iii) The fleas play only a secondary role in the matter of transmission. The infection is maintained actively in the rodents themselves.

(iv) *X. cheopis* was the vector of choice for successful continuous transmission experiment while *X. astia* could propagate infection upto the third series.

(v) In the perpetuation of infection organism of low grade virulence played more important role than the virulent organism. This virulence is usually regained by the circulation of the organism though susceptible hosts.

(vi) The non-immunised rats exposed to infection but surviving for long periods retained the infection in larger numbers than the artificially immunised ones although the majority of either of them ultimately succumbed to the infection.

(vii) Bacteraemia in the absence of impending clinical condition could not be established in these experiments as we were not sufficiently equipped to handle rats with bacteraemia before death.

(viii) It was further observed that two doses of immunisation with specific plague protein Antigen A produced better immunity than the killed Haffkine plague vaccine and *R. rattus* was found more vulnerable than *B. bengalensis*. Also, comparatively large number of non-immunised rats which survived for long periods were carrying *Y. pestis* in subclinical state than the immunised rats. The longest period observed was 180 days and the average 114 days. The final could not be reached due to the exhaustion of the experimental rats.

Final Lead to Eradication

Having equipped with the above knowledge it became very easy to handle epidemic situation and also measures for eradication. The basic point is that plague being primarily a disease of the rats attempts should be intensively directed to eliminate infection from among the rats and accordingly the entire gamut of epidemiologic work was then directed to eradicate plague infection from amongst the rat population in the twin cities of Calcutta and Howrah.

The following steps were taken:

Control and Prevention of Plague (Seal 1980, 1987)

The various measures applied for the control of plague are:

(i) The public were instructed how to handle dead rats;
(ii) Anti-rat measures against commensal rodents such as killing by mechanical means, and by use of predatory animals, trapping, fumigation, poisoning, baiting and other methods.
(iii) Rat-proofing of houses and godowns etc.
(iv) Control of wild rodents
(v) Vector control by DDT insufflation, application of other insecticides and fumigation
(vi) Direct control of bubonic plague through hospitalisation of patients, management of contacts, evacuation, mass vaccination and chemo and sero-prophylaxis
(vii) Control of pneumonic plague by case detection, isolation and treatment of patients, management of contacts, protection of staff and disinfection of contaminated objects

(viii) Disposal of the dead
(ix) Control of plague at a distance through quarantine laws and international intelligence system
(x) The State Control Organization should search for the wild rodents and carry out rat destruction measures till the foci are completely eliminated.

Precautions: Vector control by pesticides by DDT or BHC spraying operations should be intensive and complete, because incomplete spraying often leads to resistance against the pesticides. The results of vector control operations in different parts of India has been published by Wagle and Seal (1953).

Control of Pneumonic Plague

Pneumonic plague is one of the easiest to control if precautionary measures are properly undertaken at the very onset.

(i) Compulsory and complete isolation of active and suspected cases
(ii) Cases should be attended under the cover of mask and by those who had received anti-plague vaccine
(iii) Start intensive treatment with streptomycin, sulpha drugs and with highly potent anti-plague serum
(iv) It may be supplemented by the usual anti-rat measures by cyanogassing and other measures
(v) Protect others in the house and neighbourhood by administering anti-plague vaccine and chemoprophylaxis by sulpha drugs
(vi) Provide all arrangements for laboratory diagnosis and special camp hospital
(vii) Start strict surveillance operation.

Surveillance of Plague

Surveillance is indispensable for areas where natural plague foci exist or where there is history of past plague infection. The method provides sound basis for plague control programme and indicate the need for long term ecological studies in crucial areas with a view to fianlly eradicate the disease. In fact, the surveillance operation undertaken by the author supported by the experimental work on rat movement, ecological studies of rats and fleas ultimately led to the eradication of the disease from India. The surveillance operation include the following steps (Seal & Modak 1975, 1976)

(i) Trapping of rats from different areas systematically days after days and months after months and examination of samples of living and dead rats for detection of plague organism and test them for virulence
(ii) Identification of fleas, seasonal variation of density and breeding spree
(iii) Insufflation of rat burrows and disinfestation of the premises of the affected wards and areas with DDT
(iv) Regular and repeated cyanogassing of rat burrows
(v) Detection of human cases, if any, their diagnosis and treatment and associated preventive and control measure
(vi) Immunisation of persons living in the affected areas with anti-plague vaccine
(vii) Carrying out ecological investigation and other researches on rodents fleas and improvement of bacteriological technique
(viii) Carrying out health education measures particularly for eliciting cooperation of the people of the affected areas in handling dead rats and other methods of prevention

The above measures were continuously carried out through a plague control laboratory maintained for surveillance work for about 25 years till no trace of any infection among the trapped or dead rats collected daily in hundreds could be detected continuously at least for the last 10 years. In other words, the plague control laboratory was able to destroy several generations of rats in order to achieve this end. During this surveillance operation two new methods were discovered by the author namely (i) gel-precipitation test and (ii) new medium for sugar fermentation test (Seal 1972b). The gel containing specific soluble antigen and penicillin and streptomycin could identify the rats possessing immunity against plague infection indicating existence of infection among rat population. The advantage is that the test can be undertaken right in the field and hundreds of rats can be examined in a single day. Thus if the resistance test becomes continuously negative for 2 years it means absence of subclinical infection in the rat population or absence of plague infection (Seal 1972 a).

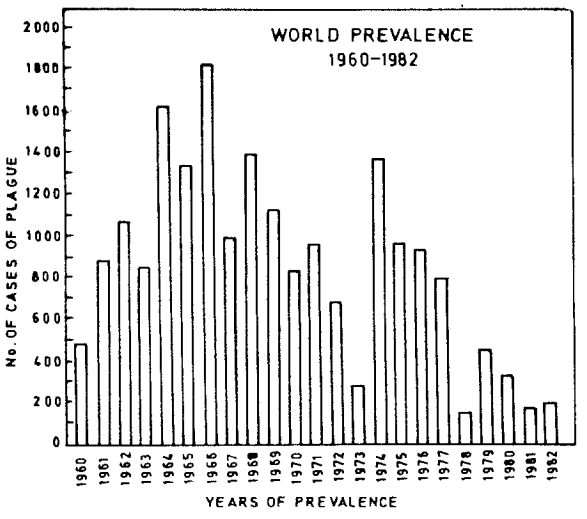
Additional Measures

During the period between 1959 and 1980 incidence of plague if reported anywhere in India was investigated under the guidance of the author e.g. in Mysore, Karnataka, Hyderabad and Assam (Gauhati) and eradication measures undertaken. As a result no plague has been reported from any area in India from 1968 onwards.

Diagnosis and Treatment of Plague (Seal 1980)

For arriving at a correct diagnosis and effective treatment the following steps are recommended:

(i) Collection of epidemiological data in a specially designed schedule by house to house visit
(ii) Thorough clinical examination with particular attention to the sets of glands affected and their nature



Figures 1

(iii) Differential count of blood in certain cases and culture of blood and bubo materials

(iv) Collection of sera from patients for serological examination.

(v) Animal inoculation test in a few instances only

(vi) Collection of dead and live rats and fleas in the houses or nearby areas and examining them for physical evidence of plague by dissection and by examination of spleen smear and culture of splenic material and blood from heart. This would provide evidence of area infection and possible involvement of human beings

(vii) Distribution of the classical features of four clinical varieties of the disease namely, (a) pestis minor, (b) bubonic, (c) septicaemic and (d) pneumonic to the field workers as well as to the hospital staff. Expecting pneumonic type might arise from the bubonic or septicaemic type the field workers should be instructed to look for the common features described in the text books.

Drugs which have been found effective for treatment are: (Seal 1960c)

	Survival rate
Streptomycin antibiotic	Not recorded
Antiplague serum (Haffkine Institute)	76.05
Sulphapyridine	73.0
Sulphathiazole	77.1
Sulphathiazole and antiserum	80.0
Sulphamerazine	97.5
Sulphadiazine	91.2

Appendix I—The latest world position of reported incidence of plague cases between 1960 and 1982 is shown in figure 16.

References

Austrian Plague Commision 1895–1900 *Denk Schr Acad Wiss Wien* **66** Part I.

Bacot A W and Martin C J 1914 *J. Hyg (camb)* **13** 209

Baker E E, Sommer H, Foster L E and Meyer K F 1947 *Proc. Soc. Exp. Biol.* **64** 139

Bezsonova 1930 *Zbl Bakt (I Abt Orig)* **119** 32

Burrows T W, and Bacon C A 1956 *Brit. J. Exp. Pathol.*, **37** 461

——, 1959 *Proc. Diamond Jubilee Haffkine Institute, Bombay*, 14

Devignat R 1951 *W H O Bull.* **4** 247

—— 1958 *Rep. presented before the W H O Expert Committee at Geneva*

Gauthier H C and Raybaund 1903 *Rev. Hyg. Paris* **23** 426

German Plague Commission 1899 *Arb Gesundo Amt Berlin* 16

Indian Plague Res. Commission 1907 *J. Hyg. Camb.* 1907 *Vols 1 to 12*

Liston, W Glen 1906 *J. Hyg. Camb.* **6** 425

Rao Raghavendra 1936 Studies in Epidemiology of plague in Calcutta with special reference to long term periodicity. DSc Thesis, Cal Univ.

Schutze H 1932 *Brit. J. Exp. pathol.* **13** 284

Seal S C 1942 *Ann. Rep. Haffkine Institute, Bombay*, 4

—— 1943 *Ann. Rep. Haffkine Institute, Bombay*, p.43

—— and Stewart S E 1941 *Pub. Hith Rep.* **56** 792

—— 1950 *Ann. Biochem. Exp. Med.* **10** 99

—— 1951 a *Ann. Biochem Exp Med.* **11** 129, 143, 171

—— 1951 b *J. Immunology* **67** 93

—— 1951 c *Proc. Soc. Biol. Med.* **77** 675

—— 1952 *Ann Biochem Exp. Med.* **12** 123, 143

—— 1953 *J. Immunol.* **71** 169

—— 1954 *Ann Biochem. Exp. Med.* **14** 9

—— 1959 Envelope and Capsule of Plague bacillus. Paper presented before the 1st Microbiol. Congr. held in Calcutta in October 1959

—— 1960a *W H O Bull.* **23** 283, 293

—— 1960b *Indian J. Med. Sci.* **14** 765

—— 1960c *Indian Med Assoc.* **35** 38

—— 1961 *Indian J. Med. Res.* **49** 1019

—— 1972a *Indian J. Med. Res.* **60** 825

—— 1972b *Indian J. Med. Res.* **60** 1414

—— 1976 *Indian J. Pub. Hlth.* **20** 25

—— 1980 D. Sc. thesis on Plague, University of Bombay

—— 1987 Plague conquest and eradication in India; ICMR Monograph 1987

—— and Bhattacharjee L M 1961a *Indian J. Med. Res.* **49** 974

—— and —— 1961b *Indian J. Med. Res.* **49** 1008

—— and Modak K K 1975 *Indian J. Pub. Hith.* **19** 126

—— and —— 1976 *Indian J. Pub. Hlth.* **20** 25

—— and Mukherje S P 1950 *Ann Biochem Exp. Med.* **10** 79

Sharif M 1951 *W H O Bull.* **4** 75

Simond P L 1898 *Ann Instt Pasteur* **12** 625

Sokhey S S 1939 *Indian J. Med. Res.* **27** 313

—— and Maurice H 1935 *Bull. Off. Int. Hyg. Pub.* **27** 534

Wagle P M and Seal S C 1953 *W H O Bull* **9** 597

Wu-Lien-Teh 1926 *A Treatise on Pneumonic Plage.* (League of Nations Publication)

—— et al. 1936 *Plague manual* (China: Shanghai)