



Report of the Haffkine Institute

For the Year 1942-43

BY

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Director, Haffkine Institute

BOMBAY

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YEARS 1942 AND 1943

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Dr. R. K. Gokhale, L.C.P. & S., S.M.S.

Plague Inquiry

Dr. S. C. Seal, M.B. (up to 31st March, 1943).
Mr. S. P. Mukherji, M.Sc. (up to 11th November, 1942).

Leptospirosis Inquiry

Dr. M. N. Lahiri, M.B. (Cal.), D.T.M. (Liverpool).

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II. General Review

The two years under review put considerable strain on the resources of the Institute. The unsettled conditions in the country and the danger of air-raids called for large quantities of different therapeutic sera to be supplied to different provinces. The recently organised department of serum production had to increase its work considerably to cope with these demands. The number of horses under immunization was increased to 151 and every effort was made to meet the excessive demands. Heavy demands were also made on the Department of Pharmacology. It rendered very valuable aid to the Army Authorities. All the pharmacopœial preparations and other medical supplies purchased by the Army in Western India or imported through the Port of Bombay, were submitted to this department for analysis. Over two thousand and six hundred samples were received and analysed. This service has helped the forces to maintain the quality of the medical supplies at a high level. Owing to the shortage of antiseptic solutions, an antiseptic solution called "Pemon" has been prepared by the Department of Chemotherapy and is being supplied to the hospitals.

Outbreak of cholera called for large quantities of cholera vaccine. Other vaccines were also in great demand. These demands were unusually high and had to be met in spite of the scarcity of agar in the country. However, the Institute managed to meet them.

Epidemics of plague broke out in certain parts of China, and the situation required immediate attention. Arrangements were made to train a large number of Chinese medical officers, sanitary engineers and bacteriologists in plague control work at the Institute, and intensive training was given in the measures adopted for the control and prevention of plague epidemics. Training was also given in the large-scale production of plague vaccine and therapeutic sera.

NEW DEPARTMENTS

The need for a separate Department of Nutrition was felt for a long time, and the prevailing conditions consequent on the present war gave the necessary impetus to organise this department. Wartime feeding of the general population created a large number of nutritional problems which required the close co-operation of the laboratory and the state. The Institute was fortunate in securing the services of Dr. M. V. Radhakrishna Rao, M.B., B.S., Ph.D., who was on the staff of the Nutrition Research Laboratories, Coonoor, for over six years and had done valuable research work on deficiency diseases. Due to the introduction of rationing in the city of Bombay, a large number of samples of food-stuffs for analyses were referred to the Institute, and also technical advice on nutritional matters was sought. The new Department of Nutrition, which had just been organised, rendered valuable aid in this sphere of work with the help of the Department of Pharmacology which conducted analytical work. This department has proved of great value even during its short existence.

The Institute also organised in collaboration with the Red Cross Society a blood bank and it processed 847·82 litres of blood since April, 1942. A Strumia type of plasma drying machine was built in the Institute from local resources and the plasma obtained from blood was preserved by drying it from the frozen state for the first time in India. The pilot plant proved quite efficacious, a moisture content of less than 1 per cent having been consistently achieved.

RESEARCH WORK

In spite of the increased pressure of routine work due to war emergency, research work was continued and valuable contributions were made. The researches conducted during the two years under review are summarized in Part II of this report, but attention is drawn here to some of the more important findings.

Plague Vaccine.—A new plague vaccine, which is much more potent and very much less toxic than the old Haffkine Vaccine, has been developed. It was found that plague organisms are grown better in casein hydrolysate instead of the mutton digest used at present. Further if the growth is treated with formalin (0.075 per cent), the toxicity is reduced still further. It is also found that broth-grown vaccine is a very stable product and does not deteriorate markedly for several years even if stored at room temperature. The new vaccine will go into production as soon as arrangements can be made for making the new broth in bulk.

Chemotherapy of Plague.—During the years under review, two field trials were carried out; first at Latur and second at Poona. At Latur where sulphathiazole, sulphapyridine and antiplague serum were tried, it was found that, as compared with sulphapyridine, sulphathiazole produces a significantly higher recovery rate among cases with severe septicaemia and that serum and sulphathiazole gave the best results. At Poona the relative merits of sulphadiazine and sulphathiazole for their curative value against plague infection were studied. Both these drugs have marked curative effects. In all, 157 cases were treated, out of which 81 cases were treated with sulphadiazine with 10 deaths, giving a case mortality of 12.3 per cent, and 76 cases were treated with sulphathiazole with 16 deaths, giving a case mortality of 21.0 per cent. Apparently, sulphadiazine appears to be more potent, but does not show any statistically significant difference as compared to sulphathiazole. However, sulphadiazine has the advantage of being less toxic. Its concentration in blood can also be more uniformly maintained.

Sulphonamide paste and war wounds.—Treatment of war wounds was investigated in the Department of Pharmacology and a technique of dressing the wounds with cod-liver-oil-sulphonamide paste and sulphanilamide impregnated pad was evolved. This new method of treatment has received an extensive clinical trial on the Burma front, and it is being adopted as a routine measure.

Immunization with Tetanus toxoid.—Experiments carried on normal persons showed that immunization with three doses of tetanus toxoid was more effective than that obtained by injecting two doses of the toxoid.

Plague Recrudescence in the Bombay Province.—The plague endemic centres in India are confined to cool and moderately damp areas comprising watersheds situated in the submontane regions and high tablelands. In Southern India, there are three such endemic centres: One in H. E. H. the Nizam's Dominion; the second one in the eastern watersheds of the Western Ghats in the Dharwar and Belgaum districts and Mysore State; the third one in the watersheds of Shevaroy, Nilgiri, Palni and Cardamom hills. Plague radiates from these endemic centres and the extent of the infected areas increases or decreases very gradually showing a secular tendency. In the Bombay Province the Sholapur and Bijapur districts are affected by the endemic centre located in His Exalted Highness the Nizam's Dominion, and Dharwar, Belgaum and North Kanara districts from the endemic centre situated in the Western Ghats.

The phenomenon of "carry-over" was studied in the Dharwar district. The plague infection is not apparent during the hot season, as its intensity is diminished and it runs a slow subterranean course from burrow to burrow. The end of the off-season is marked by the advent of the rainy season which results in a striking increase in flea index and causes rats from the outside shelters to herd into burrows within rooms and remain there perforce. A stage is thus set for the infection to flare up again.

A study of the burrows and nesting conditions of different kinds of rodents and the laboratory experiments on the nutritional requirements of the larvae of the three rat-fleas belonging to the genus *Xenopsylla*, indicate that the main factors controlling their irregular and patchy distribution are the temperature tolerance and nutritional requirements of their larval stages, which differ in the three species.

III. Department of Vaccines.

A. HAFKINE'S PLAGUE VACCINE.

During the years 1942 and 1943, 2,730,798 c.c.* and 3,830,643 c.c. of plague vaccine respectively were issued against 4,089,888 c.c. in the year 1941.

The quantities of plague vaccine in c.c. despatched monthly during the years 1941, 1942 and 1943 are detailed below :—

Month	1941	1942	1943
January	855,567	333,090	173,238
February	396,408	258,972	132,384
March	532,620	846,297	473,916
April	110,805	90,921	101,454
May	6,852	10,365	10,716
June	3,951	1,191	68,013
July	251,466	76,569	162,291
August	258,468	141,249	438,198
September	384,660	128,442	862,185
October	420,600	304,737	511,782
November	402,243	358,077	646,101
December	466,248	171,888	250,365
Total	4,089,888	2,730,798	3,830,643

*The previous practice of giving number of doses of plague vaccine is abandoned and in conformity with other vaccines hereafter the quantities of plague vaccine will be given in c.c. For conversion of doses given in previous reports, multiplication with 3 will give the quantities of plague vaccine issued in c.c.

From the opening of the Institute to the end of 1943, a total of 149,371,365 c.c. have been issued. The output of the vaccine in quinquennial periods has been as follows :—

Years				c.c.
1897-1901	..	..	..	8,601,123
1902-1906	..	..	..	10,366,611
1907-1911	..	..	..	10,752,786
1912-1916	..	..	..	13,702,098
1917-1921	..	..	..	20,003,682
1922-1926	..	..	..	21,607,908
1927-1931	..	..	..	17,787,729
1932-1936	..	..	..	19,909,176
1937-1941	..	..	..	20,078,811
1942-1943 (two years)	..	..	..	6,561,441
Total				149,371,365

The geographical distribution of the vaccine in India and outside India during the years 1941, 1942 and 1943 is given below :—

				Quantities in c.c.		
				1941	1942	1943
BRITISH INDIA						
Bombay Province	..	..	..	924,963	309,651	1,382,997
Madras Province	..	..	..	590,247	790,605	1,173,324
Bengal, Bihar and Assam	..	..	..	12,609	2 0,997	103,245
United Provinces of Agra and Oudh	..	..	..	245,103	754,956	651,621
Punjab	..	..	..	4,293	6,903	12,297
North-West Frontier Province	..	..	..	900	846	2,250
Central Provinces and Berar	..	..	..	163,386	31,992	189,135
Delhi Province	..	..	..	900	18	3,240
Sind Province	..	..	..	5,931	351	—
Port Blair	..	..	..	72	—	—
Total				1,948,404	2,096,310	3,521,109

				Quantities in c.c.		
				1941	1942	1943
INDIAN STATES						
Southern Maratha States	..	..	..	98,766	18,144	34,188
Hyderabad Deccan	..	..	..	498,141	245,934	39,096
Mysore	..	..	..	693,483	70,005	157,152
Patiala	..	..	..	612	—	1,902
Jaipur	..	..	..	216	2,700	—
Jodhpur	..	..	..	90	576	90
Indore	..	..	..	1,686	246	900
Cochin	..	..	..	144	486	—
Baroda	..	..	..	6,336	—	—
Gwalior	..	..	..	3,024	—	36
Benares	..	..	..	1,800	900	16,290
Travancore	..	..	..	—	2,412	54,180
Bhopal	..	..	..	432	5,700	2,490
Bikaner	..	..	..	—	216	—
Rewa	..	..	..	—	—	2,160
Panna	..	..	..	—	—	600
Total	..	..	..	1,304,730	347,319	309,084
FOREIGN COUNTRIES						
Burma	..	..	..	514,080	181,440	—
Federated Malaya States	..	..	..	28,728	—	—
Egypt	..	..	..	181,440	90,720	—
Singapore	..	..	..	3,174	—	—
Portuguese Government	..	..	..	—	—	450
Aden	..	..	..	3,114	15,000	—
China	..	..	..	105,840	—	—
Iraq	..	..	..	378	—	—
Total	..	..	..	836,764	287,160	450
Grand Total in c.c.	..	..	..	4,089,888	2,730,798	3,830,643

B. Other Vaccines

The following are the quantities of anti-cholera, anti-typhoid and anti-meningococcal vaccines issued during the years 1942 and 1943:—

Month	Anti-cholera Vaccine in c.c.		T. A. B. Vaccine in c.c.		Anti meningococcal Vaccine in c.c.
	1942	1943	1942	1943	1943
January ..	12,240	420,389	1,813	10,897	—
February ..	6,577	222,672	1,259	6,607	—
March ..	72,361	131,345	2,396	18,301	—
April ..	160,937	212,253	6,407	13,443	—
May ..	432,129	135,712	35,817	15,088	2,035
June ..	595,363	619,883	54,988	24,644	410
July ..	482,590	640,137	6,821	24,460	—
August ..	705,814	315,896	7,938	25,684	—
September ..	620,257	116,868	21,320	23,365	—
October ..	84,254	233,006	6,267	8,515	—
November ..	30,184	37,493	4,749	6,064	—
December ..	36,997	488,786	2,556	7,400	—
Total ..	3,149,703	3,574,540	152,331	184,468	2,505

IV. Department of Antitoxins and Sera

The department has received strong support from all over India. Its products are very much in demand by all the hospitals, but as the department has had to work under some handicaps, it could not meet all the demands. The work of building stables proceeded much slower than was expected and this made the proper care and maintenance of horses very difficult. The necessary equipment for processing sera, which had had to be ordered from abroad, has not yet arrived, and work has had to be carried out by makeshift arrangements. In spite of all this, high quality of the products was maintained. When the department is properly equipped, its production will be larger and there will be a demand for all it can produce.

1. *Horses*.—A total number of 151 horses were under processes of hyperimmunisation during these two years. Out of these, 137 were new admissions—75 during 1942 and 62 during 1943. The horses were distributed and re-distributed according to the suitability of their response against any particular antigen. More than half

the number of horses were, however, used for the production of tetanus antitoxin. The horses were grouped as follows:—

For production of	Number of horses used during 1942 and 1943
Tetanus antitoxin	87
Gas Gangrene antitoxin	21
Diphtheria antitoxin	23
Anti-dysentery serum (Shiga)	14
Anti-plague serum	2
Anti-snake-venom serum	4
Total	151

2. *Preparation of toxins.*—A large number of batches of toxins had to be prepared for immunization of the horses. The following table shows the amount of each type of toxin prepared during the years 1942 and 1943:—

Type of Toxin	Number of batches prepared		Total volume in litres	
	1942	1943	1942	1943
Tetanus	51	91	487	918
Welchii	12	11	37	36
Septique	10	11	28	29
Oedematiens	4	7	11	18
Diphtheria	17	61	68	244
Dysentery (Shiga)	8	11	36	48

3. *Toxoids.*—A few of the above batches of tetanus and diphtheria toxins were, toxoided for use in man. For facility of testing, a number of different batches were pooled together and given a mixed batch number. Two hundred and twenty litres of tetanus toxoid were prepared. The amount of tetanus toxoid in each mixed batch averaged 20 litres. Only 11 such batches were prepared during these two years, because there was no demand for supply. Each of these batches satisfied the criteria laid down by the British Therapeutic Substances Regulations and the United States Public Health Service. Three of the batches of toxoid were also tested by Sir Percival Hartley, Director, Biological Standards, National Institute of Medical Research, Hampstead, London. He corroborates the findings that the samples satisfy the requirements of the Therapeutic Substances Regulations.

Five batches of diphtheria toxoid, amounting to 25 litres were prepared during these two years. They were issued for use in man, after purifying and concentrating them by precipitation with alum.

4. *Antitoxins.*—The total number of the batches of antitoxins and sera prepared was 181 during 1942 and 203 during 1943. Each batch consisted of plasma or serum pooled from a number of horses. The volume in each batch varied from 18 to 22 litres. All excepting the natural anti-dysentery and the anti-plague sera, were concentrated and purified by the ammonium sulphate salting out method.

The following table gives the numbers of batches of different items prepared during 1942 and 1943 :—

Type of antitoxins or sera	Number of batches prepared		Total volume in litres	
	1942	1943	1942	1943
Tetanus antitoxin	110	129	330	400
Gas gangrene antitoxin	29	20	90	60
Diphtheria antitoxin	31	39	62	78
Anti-dysentery serum (Shiga).—				
(a) Natural	9	10	90	100
(b) Concentrated	2	2	6	6
Anti-plague serum	<i>Nil.</i>	3	<i>Nil.</i>	12
Total	181	203	578	656

5. *Supply.*—The following quantities of sera and toxoids were issued during the years 1942 and 1943 :—

	Number of ampoules supplied	
	1942	1943
1. Tetanus antitoxin—		
(a) Prophylactic—3,000 I.U. in 2 c.c. ..	84,685	45,998
(b) Curative—20,000 I.U. in 10 c.c. ..	8,934	10,947
(c) Highly concentrated—20,000 I.U. in 4 c.c. ..	4,550	659
(d) Diluted—1,000 I.U. per c.c. (bulk supply) ..	38,000 c.c.	104,000 c.c.
2. Anti-gas gangrene serum, in 10 c.c. ..	8,037	4,123
3. Gas gangrene antitoxin containing 3,000 I.U. Welchii, 1,500 I.U.* Septique and 1,000 I.U. Oedematiens antitoxin in 4 c.c. ..	1,548	839
4. Diphtheria antitoxin—		
(a) Prophylactic—1,000 I.U. in 2 c.c. ..	5,721	2,779
(b) Curative—10,000 I.U. in 10 c.c. ..	4,254	6,038
(c) Highly concentrated—10,000 I.U. in 5 c.c. ..	270	546
5. Anti-dysentery serum (Shiga)—		
(a) Natural—4,000 I.U. in 20 c.c. ..	3,648	4,513
(b) Concentrated—4,000 I.U. in 5 c.c. ..	939	361
6. Anti-plague serum in 20 c.c. . .	<i>Nil.</i>	529
7. Tetanus toxoid—		
(a) 10 c.c.	59	14
(b) 1 c.c.	1,002	2,384
8. Diphtheria toxoid—		
(a) 5 c.c.	103	690
(b) 1 c.c.	320	247
9. Normal horse serum	246	372

6. *Training*.—Dr. Wong Yu-wei of the National Epidemic Prevention Bureau, Kunming, China, worked in this department on the production of diphtheria and gas gangrene toxins in peptone-free medium. Mr. Benwyll Chao-Ming Hung of the North-west Epidemic Prevention Bureau, China, also worked in this department. He studied on the stability of Schick toxin at high temperature of storage.

Dr. Wong experienced a great difficulty in obtaining supplies of peptone in China. He could not prepare toxins in the absence of peptone. He, therefore, came here to work out a peptone-free medium, which would be suitable for the production of toxins. Mueller's casein digest medium is suitable for the production of diphtheria toxin. But its utility in the production of tetanus toxin and toxins of other anaerobes is still doubtful. Hence, Loiseau and Philippe's modification of Martin's stomach digest broth was selected as the medium for trial. It was found to be good for the production of all types of toxins. Very high titre toxins were obtained. In the case of diphtheria toxin production, it was found that still-better titres can be obtained by following up the peptic with the tryptic digestion. His difficulties had, therefore, been solved.

Mr. Hung studied on the stability of Schick toxin. The problem of maintenance of the potency of biological products is as acute in China as in India. This is more so in the case of diluted antigens, such as Schick toxin. He found that only desiccated Schick toxin can maintain its original potency for a long time. The dried preparation, which he had tested, remained unaltered at 37° C. for 89 days and at 45° C. for 22 days, whereas the dilutions prepared in the reputed stabilizing diluents, such as (i) a mixture of 2.7 per cent glycerin with 0.08 per cent gelatin, (ii) 1 per cent human serum, and (iii) 0.5 per cent proteose peptone (Difco) proved to be relatively unstable.

V. Department of Entomology

This department carried out a number of routine duties and researches during the two years. Investigations on the nutritional requirements of the larvae of the three common Indian rat-fleas were extended so as to include observations in the field, with a view to throw light on the irregular and patchy distribution of these fleas in India. These and previous investigations on the effects of different constant temperatures and relative humidities upon the growth of early stages of the three common Indian rat-fleas and on the male genitalia of Siphonaptera were under preparation for publication. The results of these investigations are given in the form of a summary in Part II of this report, and will be very shortly sent for publication.

The department was also engaged in conducting an inquiry into the recrudescence of plague in the Dharwar and Belgaum districts. The field work of the inquiry was brought to a close by 20th March, 1943. The staff of the inquiry was reduced and brought to the Haffkine Institute to write the report and to carry out further laboratory experiments with a view to confirm the field observations. Plague infected villages in the Sholapur, Bijapur, Belgaum and North Kanara districts during the years 1935-42 were plotted on skeleton maps, which enabled to understand the progress of plague and to determine the existence of an endemic centre in the Southern Registration Districts. This study when further extended so as to comprise the present position of plague in India led to a reasonable generalization that endemic centres are located in the watersheds of different ranges of mountains in India. The phenomenon of carry-over of plague was studied in detail at village Alnavar in the

Dharwar District. The results of the investigations of the inquiry during the two years are summarised in Part II of this report, and a detailed report is in the course of preparation for publication.

On the basis of field experience gained in connection with the Plague Recrudescence Inquiry, recommendations for organized and suitable methods of plague control were made to the Director of Public Health for the Government of Bombay.

During the two years several inquiries about the identification of fleas and other insects, and general information about insects and rodents and their control were attended to. Samples of insecticide sprays containing pyrethrum were biologically tested for a private firm and the Officer-in-charge, Ordnance Inspection Depot, Sewri. A few samples of rat-traps were received for examination and expert advice from different local bodies and firms. Suggestions for improving their construction were given to them. Two elongated improved wonder rat-traps and two cyanogas foot-pump dusters were sent to Mr. Hsu In-Dong, Representative in India for National Health Administration, China, for transmission to the Chinese Government at Chungking.

The Curator of the Bombay Natural History Society was given advice in preparation of models of various stages of a rat-flea, bed-bug and human louse and Ixodid and Argasid ticks. A number of physicians, bacteriologists, engineers and sanitary inspectors from China and India were given training in anti-plague measures and epidemiology of plague both in the Haffkine Institute and Plague Research Laboratory at Dharwar.

Mr. D. M. Munshi, B.Sc., a part time research student, worked upon the morphology of the proventricular valve of *Xenopsylla cheopis* up to 15th July 1943.

Dr. M. Sharif was at Dharwar for the following periods to supervise the work of Plague Recrudescence Inquiry in Sholapur and adjoining districts :—

1942—14th to 30th January ; 19th February to 5th March ; 23rd March to 10th April ; 6th to 25th May ; 19th to 25th June ; 9th July to 4th August ; 25th August to 10th September ; 3rd to 26th October ; 18th to 29th November ; 14th to 23rd December.

1943 - 9th to 22nd January ; 7th to 23rd February ; 8th to 21st March.

A. EXAMINATION OF BOMBAY CITY RATS

A permanent organisation is maintained by the Municipality of Bombay for catching rats. Approximately 2,000 rats are received every day by the Institute from the different wards within the Municipal area. These rats as in the previous years are either trapped alive or found dead in the various parts of the city, including the Port Trust Docks. All the rats are classified according to the species. Only the dead ones are dissected and examined for post mortem signs of plague infection. In doubtful cases smears and cultures are made. All the rats daily received are finally incinerated. Besides providing valuable information on the subject of epidemiology, this work is intended to give the municipal authorities the first signal of any plague epizootic in a part of the city with a view to detect and control plague in the city.

Tables giving the monthly totals of rats received and the number out of them that were examined are given below. During the two years under report no plague infected rat was detected, and no human plague case occurred in the city and the Bombay Port Trust Docks.

TABLE I :—Rats received at the Institute from the Bombay Municipality during 1942 and 1943

Month	1942			1943		
	Alive	Dead	Total	Alive	Dead	Total
January ..	30,769	36,676	67,445	26,975	28,272	55,247
February ..	26,339	27,232	53,571	23,217	26,664	49,881
March ..	27,731	30,871	58,602	21,119	26,970	48,089
April ..	26,072	24,224	50,296	22,746	28,715	51,461
May ..	21,140	23,311	44,451	23,076	24,852	47,928
June ..	22,726	22,397	45,123	23,739	24,563	48,302
July ..	21,641	20,947	42,588	23,332	23,803	47,135
August ..	18,369	22,551	40,911	27,181	24,732	51,913
September ..	20,207	25,644	45,851	28,637	30,322	59,009
October ..	30,471	29,581	60,052	38,683	37,360	76,043
November ..	27,264	28,579	55,843	33,973	38,554	72,527
December ..	30,215	31,883	62,098	32,682	43,963	76,645
Total ..	302,935	323,896	626,831	325,410	358,770	684,180

TABLE II :—Examination of dead Rats for Plague during 1942 and 1943

Month	Dead rats examined in 1942	Dead rats examined in 1943	Found plague infected in 1942 and 1943	
			Rattus norvegicus	Rattus rattus rufescens
January ..	20,364	19,083		
February ..	17,781	19,548		
March ..	19,079	16,636		
April ..	17,182	20,158		
May ..	16,412	15,982		
June ..	15,993	17,098	Nil	Nil
July ..	14,984	16,848		
August ..	14,797	15,403		
September ..	15,730	19,329		
October ..	21,032	20,313		
November ..	18,535	27,855		
December ..	20,659	29,180		
Total ..	212,548	237,433		

TABLE III :—Showing the number and percentage of different species

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	5,904†	24·2	4,510	20·0	3,868	18·5	4,041	18·4	3,330	18·0	3,628	17·6
<i>Rattus norvegicus</i> ..	5,587	22·0	5,588	24·7	4,917	23·5	5,501	25·0	4,074	22·1	5,185	25·2
<i>Gunomys kok</i> ..	9,729	40·3	9,924	44·0	9,821	47·0	9,948	45·2	9,116	49·3	9,403	45·8
<i>Bandicota malabarica</i> ..	301	1·2	198	0·9	144	0·7	181	0·8	133	0·7	149	0·7
<i>Mus dubius</i> ..	997	4·1	826	3·7	858	4·1	972	4·4	853	4·6	917	4·5
<i>Suncus caeruleus</i> ..	1,781	7·3	1,492	6·6	1,207	6·2	1,288	6·0	947	5·1	1,244	6·1
* Unidentifiable ..	6	0·0	19	0·1	—	—	49	0·2	40	0·2	14	0·1
† Total ..	24,305	100·0	22,557	100·0	20,905	100·0	21,980	100·0	18,463	100·0	20,540	100·0

* Rats included under this head though reported to have been caught alive, were received at the Institute dead, often

† These totals do not agree with the totals of live rats in Table I, because rats received on Sundays and holidays were

TABLE IV :—Showing the number and percentage of different species

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	4,812	21·3	4,396	21·4	3,265	19·4	3,223	16·5	3,550	18·2	3,619	16·5
<i>Rattus norvegicus</i> ..	4,592	20·4	4,746	23·1	3,915	23·3	4,768	24·5	4,154	21·3	4,913	22·0
<i>Gunomys kok</i> ..	10,826	47·9	9,057	44·2	7,493	44·5	9,161	47·0	9,342	48·0	10,471	48·7
<i>Bandicota malabarica</i> ..	165	0·7	174	0·8	95	0·6	124	0·7	141	0·7	135	0·6
<i>Mus dubius</i> ..	966	4·3	914	4·5	816	4·0	683	3·5	731	3·7	552	2·6
<i>Suncus caeruleus</i> ..	1,220	5·4	1,232	6·0	1,236	7·3	1,506	7·7	1,559	8·1	1,802	8·4
* Unidentifiable ..	—	—	—	—	—	—	27	0·1	—	—	—	—
† Total ..	22,581	100·0	20,519	100·0	16,820	100·0	19,492	100·0	19,477	100·0	21,492	100·0

* Rats included under this head though reported to have been caught alive, were received at the Institute dead, often

† These totals do not agree with the totals of live rats in Table I, because rats received on Sundays and holidays were

of rats trapped alive by Municipal Corporation, Bombay, during 1942

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
3,969	20.2	3,415	22.8	3,303	23.9	6,907	26.2	4,594	20.1	4,883	20.2	52,852	20.9
4,639	23.5	3,172	21.2	3,416	21.4	4,908	18.6	4,843	21.1	5,079	21.0	56,909	22.5
8,771	44.5	6,742	45.0	7,062	44.3	12,128	45.9	11,053	48.3	11,754	48.6	115,451	45.7
142	0.7	128	0.9	105	0.7	120	0.4	139	0.6	208	0.8	1,948	0.8
656	3.3	655	4.4	658	4.1	1,093	4.1	908	4.0	839	3.5	10,232	4.0
1,490	7.6	858	5.7	881	5.5	1,233	4.7	1,350	5.9	1,435	5.9	15,296	6.0
40	0.2	—	—	11	0.1	16	0.1	—	—	—	—	195	0.1
19,707	100.0	14,970	100.0	15,936	100.0	26,405	100.0	22,887	100.0	24,198	100.0	252,883	100.0

mutilated and even putrid.

not classified.

of rats trapped alive by Municipal Corporation, Bombay, during 1943

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
4,305	20.4	4,427	19.3	5,082	22.1	6,002	21.7	6,859	22.8	5,654	20.9	55,194	20.3
4,814	22.9	5,802	25.4	5,440	23.7	5,904	21.4	6,555	21.7	6,349	23.4	61,952	22.7
9,326	44.3	10,019	43.8	10,196	44.4	13,046	47.2	14,806	47.5	12,730	47.0	125,973	46.3
174	0.8	131	0.6	130	0.6	629	2.3	357	1.2	620	2.3	2,875	1.1
646	3.1	905	4.0	923	4.0	870	3.1	752	2.5	609	2.6	9,457	3.5
1,789	8.5	1,570	6.9	1,205	5.2	1,147	4.1	1,288	4.3	1,029	3.8	16,583	6.1
7	0.0	—	—	—	—	50	0.2	—	—	—	—	84	0.0
21,061	100.0	22,854	100.0	22,976	100.0	27,648	100.0	30,117	100.0	27,081	100.0	272,118	100.0

mutilated and even putrid.

not classified.

TABLE V:—Showing the number and percentage of different

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	4,518	17.4	3,494	16.5	2,863	13.6	2,596	13.2	2,721	14.3	2,555	13.8
<i>Rattus norvegicus</i> ..	5,974	23.0	5,626	26.6	5,864	27.9	5,059	28.7	5,268	27.6	5,224	28.3
<i>Gunomys kok</i> ..	7,240	27.8	6,724	31.7	7,956	37.8	7,527	38.2	7,195	37.7	7,108	38.5
<i>Bandicota malabarica</i> ..	363	1.5	244	1.2	199	0.9	166	0.9	173	0.9	201	1.1
<i>Mus dubius</i> ..	2,107	8.1	1,572	7.4	1,368	6.5	1,169	5.6	955	5.0	773	4.2
<i>Suncus caeruleus</i> ..	2,683	10.3	1,933	9.1	1,375	6.6	1,321	6.7	1,217	6.4	1,363	7.4
Unidentifiable ..	172	0.7	121	0.6	129	0.6	125	0.6	162	0.8	132	0.7
Putrid ..	2,920	11.2	1,470	6.9	1,276	6.1	1,204	6.1	1,392	7.3	1,102	6.0
* Total ..	25,977	100.0	21,184	100.0	21,030	100.0	19,707	100.0	19,083	100.0	18,458	100.0

*These totals do not agree with the totals of dead rats in Table No. I, because rats received on Sundays and holidays

TABLE VI:—Showing the number and percentage of different species

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i>	8,769	17.1	3,826	17.0	3,006	15.9	3,317	14.1	3,191	16.8	3,004	14.9
<i>Rattus norvegicus</i> ..	5,608	25.5	5,705	25.4	5,018	26.0	5,850	24.9	4,759	25.1	5,385	26.6
<i>Gunomys kok</i> ..	7,501	34.2	8,037	35.8	6,848	35.6	9,086	38.6	6,631	35.0	7,358	36.4
<i>Bandicota malabarica</i> ..	327	1.5	270	1.2	170	0.9	251	1.1	173	0.9	186	0.9
<i>Mus dubius</i> ..	1,739	7.9	1,561	7.0	1,406	7.3	1,508	6.4	1,088	5.7	1,020	5.1
<i>Suncus caeruleus</i> ..	1,504	7.1	1,490	6.7	1,478	7.7	1,987	8.5	1,459	7.7	1,557	7.7
Unidentifiable ..	140	0.7	149	0.7	128	0.7	138	0.6	140	0.7	145	0.7
Putrid ..	1,305	6.0	1,382	6.2	1,139	5.9	1,363	5.8	1,537	8.1	1,557	7.7
* Total ..	21,952	100.0	22,426	100.0	19,253	100.0	23,508	100.0	18,078	100.0	20,212	100.0

*These totals do not agree with the totals of dead rats in Table No. I, because

species of rats found dead in the city of Bombay during 1942

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
2,889	16.4	2,998	17.2	2,940	16.2	4,452	18.3	3,998	18.2	3,785	15.6	39,809	16.0
4,726	26.8	4,529	25.9	4,746	26.2	6,209	25.5	5,223	23.7	5,821	24.0	64,869	26.0
6,097	34.6	5,961	34.1	6,535	36.0	8,013	32.9	7,006	31.8	8,568	35.3	85,930	34.5
190	1.1	164	0.9	131	0.7	234	1.0	249	1.1	411	1.7	2,725	1.1
952	5.4	1,070	6.1	1,276	7.0	1,972	8.1	1,919	8.7	1,936	8.0	17,009	6.8
1,576	8.9	1,403	8.0	1,338	7.4	1,881	7.7	1,888	8.6	1,745	7.2	19,723	7.9
130	0.8	125	0.7	102	0.6	152	0.6	140	0.6	138	0.5	1,628	0.6
1,058	6.0	1,210	7.1	1,070	5.9	1,433	5.9	1,601	7.3	1,874	7.7	17,619	7.1
17,618	100.0	17,469	100.0	18,138	100.0	24,346	100.0	22,024	100.0	24,278	100.0	249,312	100.0

were not classified.

of rats found dead in the City of Bombay during 1943

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
3,183	16.0	3,062	16.4	3,868	16.7	3,529	14.3	5,177	15.8	5,501	15.6	44,483	15.8
4,890	24.6	4,470	23.9	5,819	25.1	5,928	24.0	8,258	25.1	8,915	25.3	70,014	25.2
7,447	37.4	6,267	33.5	8,086	34.8	8,688	35.2	11,576	35.2	11,711	33.2	99,235	35.3
150	0.8	185	1.0	203	1.1	332	1.3	405	1.2	439	1.2	3,145	1.1
1,037	5.2	1,203	6.4	1,141	4.9	1,670	6.7	2,204	6.7	2,358	6.7	17,935	6.4
1,855	9.3	2,177	11.7	2,197	9.5	2,763	11.2	2,892	8.7	2,740	7.8	24,105	8.6
141	0.7	126	0.7	152	0.7	166	0.7	235	0.7	262	0.7	1,931	0.7
1,188	6.0	1,187	6.4	1,682	7.2	1,635	6.6	2,179	6.6	3,356	9.5	19,510	6.9
19,891	100.0	18,677	100.0	23,208	100.0	24,711	100.0	32,926	100.0	35,276	100.0	281,018	100.0

rats received on Sundays and holidays were not classified.

B. EXAMINATION OF RATS FROM

These rats were received along with those of the other wards of the Bombay in previous tables. A separate record of these rats giving the number and percentage Commissioner. The following tables give summaries of these records.

TABLE VII:—*Showing the number and percentage of different species*

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	441	48.8	421	49.1	448	50.7	385	45.2	503	51.0	840	62.6
<i>Rattus norvegicus</i> ..	56	6.2	52	6.1	43	4.9	87	10.2	39	4.0	56	4.2
<i>Gunomys kok</i> ..	156	17.2	134	15.6	162	18.5	191	22.4	203	20.5	225	16.8
<i>Bandicota malabarica</i> ..	18	2.0	6	0.7	8	0.9	10	1.2	17	1.7	7	0.5
<i>Mus dubius</i> ..	167	18.5	162	18.9	172	19.7	151	17.7	160	16.2	171	12.7
<i>Suncus caeruleus</i> ..	66	7.3	82	9.5	46	5.3	28	3.3	64	6.5	43	3.2
Unidentifiable ..	—	—	1	0.1	—	—	—	—	1	0.1	—	—
Total ..	904	100.0	858	100.0	874	100.0	852	100.0	987	100.0	1,342	100.0

Rats received on Sundays and

TABLE VIII:—*Showing the number and percentage of different species*

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i>	370	48.4	451	49.8	282	40.5	230	33.3	394	46.2	425	44.2
<i>Rattus norvegicus</i> ..	49	5.8	16	1.7	23	3.3	28	4.1	95	11.1	60	6.2
<i>Gunomys kok</i> ..	194	22.8	163	18.0	166	23.9	181	26.2	103	12.1	230	23.9
<i>Bandicota malabarica</i> ..	9	1.1	18	2.0	10	1.4	18	2.6	11	1.3	10	1.0
<i>Mus dubius</i> ..	181	21.2	191	21.1	162	23.3	177	25.7	192	22.5	187	19.5
<i>Suncus caeruleus</i> ..	49	5.7	67	7.4	53	7.6	56	8.1	58	6.8	50	5.2
Unidentifiable ..	—	—	—	—	—	—	—	—	—	—	—	—
Total ..	852	100.0	906	100.0	696	100.0	696	100.0	853	100.0	962	100.0

Rats received on Sundays and

THE BOMBAY PORT TRUST DOCKS

Municipality and have been already accounted for in the monthly totals given of the different species is also maintained at the instance of the Public Health

of rats caught alive from the Bombay Port Trust area in 1942

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
526	48.6	550	52.8	564	57.8	575	52.6	475	49.9	503	58.8	6,232	52.7
93	9.0	49	4.7	70	7.2	58	5.3	98	10.3	33	3.9	739	6.3
228	21.1	199	18.9	149	15.3	191	17.5	115	12.1	114	13.3	2,067	17.5
10	0.9	16	1.5	6	0.6	11	1.0	6	0.6	9	1.0	124	1.0
100	14.8	168	16.0	139	14.3	202	18.5	186	19.5	148	17.3	1,986	16.8
61	5.6	64	6.1	47	4.8	56	5.1	72	7.6	49	5.7	678	5.7
—	—	—	—	—	—	—	—	—	—	—	—	2	—
1,083	100.0	1,052	100.0	975	100.0	1,093	100.0	952	100.0	856	100.0	11,828	100.0

holidays were not classified.

of rats caught alive from the Bombay Port Trust area in 1943

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
491	52.8	299	36.9	370	45.8	364	48.4	592	56.8	340	51.3	4,614	46.2
28	3.0	43	5.3	24	3.0	53	7.0	51	4.9	25	3.7	495	5.0
155	16.7	238	29.4	181	22.4	153	20.3	180	17.3	96	14.3	2,040	20.4
6	0.6	13	1.6	7	0.8	8	1.1	13	1.2	6	0.9	129	1.4
190	20.4	186	22.9	193	23.9	107	14.2	153	14.7	161	23.9	2,080	20.8
60	6.5	32	3.9	33	4.1	67	8.9	53	5.1	40	5.9	613	6.2
—	—	—	—	—	—	1	0.1	—	—	—	—	1	0.0
930	100.0	811	100.0	808	100.0	753	100.0	1,042	100.0	674	100.0	9,977	100.0

holidays were not classified.

C. EXAMINATION OF RATS FROM STEAMERS AND OTHER SOURCES

During the years 1942 and 1943, 1,301 and 1,182 dead rats and 6 and 19 smears from dead rats respectively were received from different towns of the Bombay Province. As a result of their examination, 2 rats were reported upon as being plague infected in 1942 and 7 in 1943 from towns as shown in the following table :—

TABLE IX :—*Showing the different localities in the Bombay Province from which plague infected rats were received during the years 1942 and 1943*

Name of Town	Number of plague infected rats in 1942	Number of plague infected rats in 1943
Bhatkal	1	1
Karwar	1	1
Poona	1	5
Total ..	2	7

Three smears from bubo puncture were received for examination for plague in 1942, and out of these two (1 from Bhatkal and 1 from Poona) were found positive to plague.

During the year 1943, the Health Officer of the Port of Bombay sent 435 killed and 3 alive rats from steamers. Out of the killed rats, 401 belonged to the species *Rattus rattus rufescens*, 1 to *Rattus norvegicus* and 33 to *Mus dubius*. All the three live rats were *Rattus rattus rufescens*. They were examined for plague and none of them was found plague infected.

D. TRAINING OF HEALTH PERSONNEL

Following the public scare on the outbreak of two plague epidemics in certain areas of China, where plague had never been previously recorded, Dr. P. Z. King, Director General, National Health Administration, Chungking, on the suggestion of Generalissimo Chiang Kia-shek, approached the Director General, Medical Services in India to make arrangements for the training of Chinese medical officers, sanitary engineers, and bacteriologists in plague control work, sera and vaccine production and other routine laboratory procedures at the Haffkine Institute. In all 24 officers listed below were given training during the two years under report for the period noted against their names.

Drs. T. H. Shen and T. H. Yieh, who were awarded scholarships by the Rockefeller Foundation were given extensive training both in the Haffkine Institute and Plague Research Field Laboratory at Dharwar for three months.

In view of the further urgent need of plague control workers to cope with the then existing plague situation in China, Dr. P. Z. King, approached this Institute through the Director General, Indian Medical Services to train 9 medical officers, 5 sanitary engineers and 6 bacteriologists, deputed by the Chinese Government. They were given a thorough training both in laboratory and field work for about three months in two batches. Various public organizations in this province also made arrangement for demonstration of their routine work to the Chinese officers. They were also helped in the purchase of apparatus, chemicals, etc.

Mr. Hung Chao-ming who was awarded a scholarship by the Government of India, has undergone an extensive course of training in bacteriology in the Department of Antitoxins and Sera.

Dr. Wong Yu-wei, Head of the Serum Department, National Epidemic Prevention Bureau, Kunming, came to the Institute on a Rockefeller Foundation's fellowship to work out some of the difficulties he had experienced in his work in China for lack of adequate laboratory facilities. He worked on them for about 9 months. Details of his work are given earlier in this report.

1. Dr. Shen Ting-hung, National Epidemic Prevention Bureau, Kunming, Yunnan, from 29th April to 1st August, 1942.
2. Dr. Yieh Tien-hsin, Department of Laboratory Medicine, Emergency Medical Service Training School, Tuyunkwan, Kneiyang, from 29th April to 1st August, 1942.
3. Dr. Cha Liang-chung, National Health Administration, from 3rd November 1942 to 31st January, 1943.
4. Dr. Shih Mao-nien, Field Director, Weishengshu, Anti-epidemic Corps, from 3rd November, 1942 to 31st January, 1943.
5. Dr. Su Teh-lung, Instructor, Public Health Personnel Training Institute, from 3rd November, 1942 to 31st January, 1943.
6. Dr. Yiu Mei-chu, Chief, Weishengshu Anti-epidemic Unit, from 3rd November 1942 to 31st January, 1943.
7. Dr. Li Ching-chieh, Technical Expert, Army Anti-epidemic Corps, from 3rd November, 1942 to 31st January, 1943.
8. Dr. Chen Yu-lien, Instructor, Emergency Medical Service Training School Ministry of War, from 3rd November, 1942 to 31st January, 1943.
9. Dr. T'Sai Fang Chien, National Health Administration, from 3rd November, 1942 to 31st January, 1943.
10. Miss Chen Hsin-mei, Bacteriologist, North-West Epidemic Prevention Bureau, from 3rd November, 1942 to 31st January, 1943.
11. Mr. Fang Keng, Assistant, Department of Bacteriology and Immunology, Army Medical College, from 3rd November, 1942 to 31st January, 1943.
12. Mr. Liu Chih-yang, Sanitary Engineer, National Health Administration, from 3rd November, 1942 to 8th January, 1943.
13. Mr. Chen Yi-mao, Sanitary Engineer, Army Medical Administration, from 3rd November, 1942 to 8th January, 1943.
14. Mr. Wang Chia-chen, Sanitary Engineer, Kweichow Provincial Health Administration, from 3rd November, 1942 to 8th January, 1943.
15. Dr. Chen Cheng-jen, Bacteriologist, Hunnan Provincial Hygienic Laboratory, from 10th February to 30th April, 1943.
16. Dr. Chen Tsung-hsien, Bacteriologist, Weishengshu Anti-epidemic Corps, National Health Administration, from 10th February 1943 to 30th April, 1943.
17. Dr. Chu Chi-ming, Assistant Technical Expert, National Central Epidemic Prevention Bureau, from 10th February to 30th April, 1943.
18. Dr. Lin Fei-ching, Instructor, Department of Bacteriology, Emergency Medical Service Training School, Ministry of War, from 10th February to 30th April, 1943.

19. Dr. Shih I-hsin, Medical Officer, Weishengshu Anti-epidemic Corps, National Health Administration, from 10th February to 30th April, 1943.
20. Dr. Fang Ching, Medical Officer, Weishengshu, Anti-epidemic Corps, National Health Administration, from 10th February to 30th April, 1943.
21. Mr. Koo Chi-tung, Sanitary Engineer, Weishengshu, Anti-epidemic corps, from 10th February to 11th April, 1943.
22. Mr. Shong Chien-keng, Instructor of Department of Sanitary Engineering, Emergency Medical Service Training School, Ministry of War, from 10th February to 11th April, 1943.
23. Mr. Hung Chao-ming, Bacteriologist, National North-West Epidemic Prevention Bureau, joined on 5th November, 1943 and still working.
24. Dr. Wong Yu-wei, Senior Technical Expert and Head of the Serum Department, National Epidemic Prevention Bureau, Kunming, China, joined on 31st July 1943 and still working.

The 1942 and 1943 classes of sanitary inspectors, health visitors and Ayurvedic and Unani subsidised medical practitioners were given short courses of training in rat examination and rat and flea destruction. In addition 36 sanitary inspectors deputed by the Director of Public Health for the Government of Bombay and local bodies were given training individually in inoculation technique and anti-plague measures for about a fortnight.

The following medical officers were deputed by their respective Governments for training in different public health problems for the periods noted against their names :—

1. Mr. A. Y. Patel, L.C.P.S., Epidemic Medical Officer, from 19th to 24th January, 1942.
2. Mr. J. R. Joshi, L.C.P.S., Sub-Assistant Surgeon in charge Port Dispensary, Okha, Baroda, from 27th May to 10th June, 1942.
3. Mr. M. M. Ashar, Epidemic Medical Officer, from 5th to 12th October 1943.
4. Mr. P. B. Badwaik, B.Sc., L.M.P. (C. P.) (given training in clinical biochemistry, bacteriology and diagnostic work), from 16th July to 16th October, 1943.
5. Dr. Kanwal Chander, M.B.B.S., L.R.C.P., M.R.C.P., Assistant Surgeon of the Hyderabad Civil Medical Department (given training in routine biochemical, bacteriological and pathological work), from 28th October to 18th December, 1943.
6. Dr. A. Nimalsuria, M.B., M.R.C.P., Medical Officer, deputed by the Ceylon Government for a course of training in Medical Research including bacteriology, from April to September, 1943.

VI. Department of Clinical Pathology

A. BACTERIOLOGICAL SECTION

The department examined clinical material from the various hospitals in the city and the mofussil places in the province. Private medical practitioners also contributed a good number of samples for diagnostic work, which were done at a very moderate cost. Free diagnostic aid is given to all practitioners in infectious diseases such as, diphtheria, dysentery, cerebro-spinal fever, enteric fever, typhus fever,

cholera, malaria, etc. The following table gives an idea of some of the important examinations carried out during the years 1942 and 1943 :—

Nature of examination	1942		1943	
	Number of samples examined	Number of positive samples	Number of samples examined	Number of positive samples
Wassermann test	6,822	1,148	7,934	1,585
Kahn test	4,117	765	1,710	630
Widal test	811	268	1,866	345
Agglutination test for typhus	15	6	500	37
Agglutination test for Brucellosis	22	2	17	1
Sputum and urine for T. B. by smear and animal inoculation	108	21	137	43
C. S. F. smear, culture, etc.	215	29	96	10
Throat swabs for diphtheria	95	9	40	17
Stools examination for intestinal pathogens ..	377	160	564	200
Nasal smears for leprosy	71	33	92	37
Friedman's test for pregnancy	71	27	152	49

A marked increase in the number of Wassermann tests is mainly due to a large number of samples sent by the Blood-Bank Centre, located in the Institute. There was also a great demand for blood grouping sera on account of blood transfusion services organised all over this and other provinces. About 500c.c. of such sera were supplied during the two years. During the year 1943, there was a small outbreak of cholera in and around Bombay. Out of 113 samples of faeces examined 31 samples were positive for *V. cholerae*. In addition to the routine diagnostic tests, this department was also engaged in preparation of autogenous vaccines, extraction of venom from poisonous snakes and in examination of samples of disinfectants for the Rideal-Walker test.

B. SECTION OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY

This section mainly examined samples of waters for their fitness for drinking and industrial purposes, samples of milk and ghee for detection of adulteration, samples of milk for the degree of cleanliness and for *B. coli* content, and samples of curd and whey for the types of lacto-organisms contained in them. Besides, a number of samples of insecticidal sprays were tested for their toxicity against adults and larvae of mosquitoes.

The following table gives a summary of various examinations carried out :—

Samples examined		Nature of examinations done		Number of samples examined
Water	..	.. Fitness for drinking purposes	..	67
Water	..	.. Fitness for swimming purposes	..	39
Water	..	.. Fitness for industrial purposes	..	3
Milk	..	.. For <i>B. coli</i> contents	..	13
Milk	..	.. For adulteration	..	5
Ghee	..	.. For adulteration	..	4
Curd	..	} For types of lacto-organisms present	..	{ 5
Whey	..			
Insecticidal sprays	..	.. For toxicity against the adults and larvæ of mosquitoes	..	33

VII. Department of Pharmacology

The activities of the department were largely influenced by the present emergency. Early in 1942, the department was reorganised to enable it to undertake testing work for the military authorities. When in 1943, the department was taken over by the Government of Bombay, the military authorities continued to bear a part of the cost of the department. Even the research of the department was largely devoted to war effort.

1. *Examination of drugs, chemicals and apparatus on behalf of the military authorities.*—This work was begun in April 1942. The samples were sent by the Officer Commanding, Medical Stores Inspection Depôt. They consisted chiefly of British Pharmacopœial drugs, chemicals, apparatus, surgical dressings, glassware for alkalinity tests, injectable preparations for sterility tests, mosquitocidal sprays and pastes, etc. In 1942, the number of samples examined was about 1,000 and this increased to more than 1,600 in 1943. The impression one gets from these examinations is that adulteration of drugs and the marketing of drugs of low potency are quite common.

2. *Preparation of sulphonamide pastes and pads for the use of the army.*—Some research work done in this department about four or five years ago had attracted the attention of Army Medical authorities and they approached the department with a specific problem of prevention of infection in war-wounds. This problem was investigated experimentally and the research work done in this connection with collaboration of army authorities is referred to in another section. The result of these investigations was the elaboration of a technique which is now undergoing an extensive clinical trial in the field. Preliminary reports show that the technique is highly successful. The technique consists in applying to the wound a specially made cod-liver-oil-sulphonamide paste through a rubber nozzle attached to a collapsible tube and dressing the wound with a sulphanilamide impregnated cotton-wool pad borne on an elastoplast. About 15,000 such tubes and nearly 8,000 special occlusive

dressings have been prepared and sent to the army for a large-scale experiment. This has entailed considerable amount of work, as no special apparatus or machinery could be obtained for large-scale preparation of these tubes and pads.

3. *Preparation of glucose saline for use of the army and A. R. P. organisations.*—Large-scale production of glucose saline was undertaken by the department. There is an acute scarcity in this country of alkali-free glass and a search was to be made to find suitable containers. A very large number of bottles were examined and it was discovered that gin bottles of a particular make bearing certain numbers were made of alkali-poor glass and were suitable for storing glucose saline. Such empty bottles were procured from shops widely scattered over the city and a sufficient number collected to meet the demands. Nearly 3,500,000 c.c. of glucose saline have so far been supplied to the various organisations.

4. *Preparation of morphine solutions for the A. R. P. organisations.*—The Government of India had approached this department to prepare and examine a formula for making sterile morphine solutions which would be stable. The experimental work done in this connection became very useful when the provincial A. R. P. organisations found it necessary to equip their First-Aid Posts with this important drug. The department undertook to prepare the stabilized solutions of morphine and met all the demands that came from the A. R. P. organisations. Sufficient quantity for about 4,000 casualties was immediately prepared and arrangements were made to supply larger quantities when asked for.

5. *Standardization of plasma and serum sent by different Blood Bank centres.*—Arrangements have been made in the department to test the plasma and serum samples sent by various Blood Bank centres in the country. The test employed is a biological one, and consists in producing experimental shock in animals and treating them by injecting intravenously the plasma or serum sent for examination. The work was started by the middle of 1943 and most of the samples examined so far were those prepared at the Haffkine Institute. Some centres like those in Nagpur and Karachi have also taken advantage of this special facility and have sent their samples for examination.

6. *Miscellaneous work.*—Medical men in the army have referred some of their problems to this department for investigation. Examinations like testing suitability of certain spinal anaesthetics or determining toxicity of some organic arsenicals whose purity was doubted were made for them. At the request of the Government of India, a new anaesthetic "trichlorethylene" was fully investigated with a view to find its suitability as a general anaesthetic.

Introduction of rationing in Bombay put an extra burden on the activities of the department. A large number of samples were sent to the department for analysis and report. These consisted of raw and cooked materials prepared from different cereals which are rationed. Thus several samples of bread, cakes, "jilebi", "amriti", "puranpoli", "shankarpali", etc. were sent to detect whether they contained any wheat flour. Grains sampled from different stores in the city were examined to find whether they were fit for human consumption. The question of adulteration of wheat flour and presence or absence of wheat bran in wheat flour was frequently referred to this department for investigation. The department was also called upon to advise the Rationing authorities about the supply of sugar to firms which had applied for it to prepare essential medicines.

The present emergency has markedly stimulated the pharmaceutical industry and growth of this industry is reflected in the marked increase in the number of samples sent for examination and assay. These samples consisted mostly of galenicals, vitamin preparations and hormones. Other preparations like mosquitoicidal sprays

were also received from commercial firms for examination and assay. Examinations like determining the pH, acidity, sterility, absorbancy of cotton-wool, adulteration of quinine, etc. were also made. Besides these, the usual examination of "snake-bite cures" was also carried out.

7. *Research work.*—Besides the experimental work on treatment of wounds by the cod-liver-oil-sulphonamide paste and sulphanilamide pads, other researches undertaken by the department mainly dealt with the problem of malaria. The department received a grant from the I. R. F. A. for research work on malaria. Several drugs prepared by the Department of Chemotherapy of the Haffkine Institute were tried experimentally in monkey malaria and two of them, viz. (1) N¹-methyl-sulphathiazole and (2) 2-N¹-sulphanilamido-5-isopropylthiazole were found to have a good antimalarial activity. The problem of immunity in monkey malaria was continued and previous observations that the immunity is both cellular and humoral were confirmed. Experimental work on the chemotherapy of plague was also carried out by the department and several drugs prepared by the Department of Chemotherapy were tried in experimental plague infections in mice.

In addition, the department worked on the chemotherapy in intestinal diseases and the study of the actions of various ions on the central nervous system. The results of these investigations are given in Part II of this Report.

VIII. Department of Chemotherapy

This department is engaged in the synthesis and study of new compounds of the sulphanilamide group and also in the investigation of the best processes of preparation of some important drugs. Most of the equipment necessary for the large-scale production of sulphathiazole, sulphadiazine and atebine have been obtained and it is hoped that the production of these drugs will commence at an early date with the completion of the laboratory under construction.

Six patents have so far been taken out for the processes of preparation of some sulphanilamide derivatives and their intermediates. A new process for the manufacture of sulphathiazole has been discovered and patented. This method has, to a great extent, simplified the whole process; the advantages in this new process consist in avoiding the use of the costly and unobtainable pyridine and shortening considerably the whole working time. As complimentary to this, a very good and rapid method of production of 2-aminothiazole and 2-amino-5-alkylthiazoles which are the intermediates in the synthesis of sulphathiazole and 5-alkylsulphathiazoles respectively, has been worked out and patented. Suitable methods and practical details for the large-scale production of sulphadiazine and the methyl derivatives are being investigated. An antiseptic solution called "Pemon" has been prepared and is being supplied to the hospitals. A good and cheap method for the preparation of large quantities of phenyl mercuric nitrate has been worked out. Sulphathiazole and solutions of sodium sulphathiazole and sulphadiazine were prepared and supplied for clinical trials.

To elucidate the relationship existing between chemotherapeutic action and chemical constitution in the sulphonamide group, an additional series of derivatives of sulphathiazole, sulphapyridine and sulphathiazoline have been prepared. Attempts are being made to synthesise and study all possible sulphonamide derivatives of the thiazole group to try if compounds better than sulphathiazole could not be discovered. Of the three sulphanilamide derivatives of thiazole theoretically possible, only 2-isomer (sulphathiazole) is so far known and studied extensively. To fill up this gap, the synthesis of derivatives of the other two series so far unknown—i.e. the 4- and 5-sulphanilamidothiazoles was undertaken. This work was attended with

some difficulties because the starting 4-amino and 5-aminothiazoles were not known. After considerable exploratory work in this direction, a suitable general method for the synthesis of 4-amino and 5-aminothiazoles has been worked out and two derivatives of 5-sulphanilamidothiazole have been prepared so far.

4: 4'-Diaminodiphenyl sulphone, which once attracted attention by its high antistreptococcal activity, is again the centre of much interest because some of its derivatives show definite therapeutic action in experimental tuberculosis in guinea-pigs. Synthesis and study of new compounds of this group have been undertaken. In the first instance, a number of derivatives of 4-nitro and 4-amino diphenylsulphone have been prepared. These compounds are also of interest in extending the interesting theory of Bell and Roblin regarding the mechanism of action of the sulphonamides.

The processes of manufacture of vitamin C (l-ascorbic acid) and vitamin B₁ (thiamin) are being investigated. The number of methods described in chemical and patent literature are being carefully examined. A good method for the construction of the pyrimidine half of thiamin appears to have been arrived at. Regarding ascorbic acid, a good method of preparing the starting sugar, 1-xylose, in quantity has been worked out.

IX. Department of Biochemistry

This department has continued to give useful help to hospitals and medical practitioners in the various biochemical examinations necessary for the diagnosis of disease. Other departments of the Institute also find this department of a great help in their research work.

1. *Nutritional work*.—Investigations in connection with nutritional problems, such as the possibility of "Brab Palm" fruit as a substitute for "Red Palm" fruit, of Dalda as a substitute for ghee have been carried out.

2. *Broth experiments*.—Experimental work in connection with the improvement of the meat digest media used in the manufacture of plague vaccine in the Institute has been continued.

3. *Blood protein fractions in immunization*.—Study of the changes in the protein fractions of blood plasma of horses undergoing a course of hyperimmunization against tetanus toxin was undertaken.

4. *Respiration of plague bacillus*.—Oxidation of a series of different substrates including carbohydrates, amino acids and organic acids, is being studied by means of Warburg Manometric technique, using different virulent and avirulent strains of the plague bacillus. Effect of some drugs on the respiration of the bacillus is also being studied.

5. *Routine examinations*.—The number of routine examinations carried out in the department in the period was 3,220; bloods 1,326, urines 498, cerebrospinal fluids 1,107, faeces 51, milk 12, peritoneal and other fluids 8 and 218 special tests, such as 91 gastric analyses, 42 blood urea clearance tests, 47 glucose tolerance tests, 34 basal metabolic rate determinations, 1 digestibility of gluten determination and 3 vitamin assays. The routine examinations were mostly determinations of non-protein nitrogenous constituents such as urea, uric acid and creatinine, sugar, serum calcium, haemoglobin, van den Bergh reaction, blood proteins, cholesterol, blood sedimentation rate, Lunge's colloidal gold reaction, etc.

X. Department of Nutrition and Experimental Pathology

A. NUTRITION SECTION

This section was organised at the Institute some time ago with the object of making it a centre for research and dissemination of knowledge on human nutrition in this province. On account of the prevailing conditions consequent on the present war, the subject of nutrition is receiving increasing attention.

Since the introduction of rationing of food-stuffs in Bombay, the Institute has been called upon by Government to undertake advisory work on the various questions on human nutrition. In addition, the section has been analysing institutional diets, jail diets, etc., and giving technical advice in matters of practical nutrition.

The Government of Bombay has deputed Dr. M. V. Radhakrishna Rao to attend the meeting of the Nutrition Advisory Committee of the Indian Research Fund Association, held in New Delhi, in December 1943. A scheme for the study of "The role of nutritional factors in hepatic cirrhosis" has been submitted to the Indian Research Fund Association, for financial support. This is a new approach to the study of deficiency diseases, which combines both clinical and experimental work. If the enquiry is sanctioned, it is proposed to commence work as soon as possible.

B. PATHOLOGY SECTION

As in previous years, the main work of the section has been to study the pathological material received from different hospitals in the Province and report histological diagnosis of the morbid condition. A rough classification of the morbid histological condition of the tissues received during the years 1942 and 1943 and their number are given in the following table:—

TABLE I :—*Showing the number of pathological tissues received and examined at the Institute during the years 1942 and 1943*

	1942	1943
I. Neoplasms		
(a) Malignant tumours		
(1) Carcinoma primary		
Squamous-cell carcinoma	.. 14	21
Basal-cell carcinoma	.. 2	3
Adenocarcinoma	.. 7	4
Carcinoma simplex	.. 10	10
Carcinoma papillary	.. 1	1
Carcinoma embryonal	.. 1	1
(2) Carcinoma secondary	.. 9	9

TABLE I—*contd.*

		1942	1943
1. <i>Neoplasms</i> — <i>contd.</i>			
(a) <i>Malignant tumours</i> — <i>contd.</i>			
(3) Sarcoma			
Fibrosarcoma	..	2	5
Osteosarcoma	..	1	—
Chondrosarcoma	..	—	1
Leiomyosarcoma	..	—	1
Liposarcoma	..	—	1
(4) Malignant Lymphoma			
Lymphosarcoma	..	3	3
Hodgkin's disease	..	1	2
Lymphoblastoma	..	—	1
(5) Chorionepithelioma			
	..	—	1
(b) <i>Non-malignant tumours</i>			
Fibroma	..	2	2
Fibromyoma	..	11	18
Fibroadenoma	..	4	11
Lipoma	..	1	2
Myxoma	..	1	—
Hemangioma (capillary)	..	3	1
Hemangioma (cavernous)	..	3	—
Adenoma	..	2	2
Adenomyoma	..	1	3
Adamantinoma	..	1	—
Chondroma	..	—	1
Polypus	..	—	14
Neurofibroma	..	—	1
Osteoma	..	—	2
Papilloma	..	—	8
Naevus	..	—	1
Cystadenoma	..	—	4
Glioma	..	—	1
(c) <i>Mixed tumours</i>			
	..	2	1
(d) <i>Endothelioma</i>			
	..	—	1
Rhinosporidiosis			
	..	—	1

TABLE I—*concl'd.*

	1942,	1943
<i>II. Tuberculosis</i>		
Lymph nodes	7	14
Breast	1	—
Male genital tract	2	—
Female genital tract	6	6
Ankle	1	—
Foot	1	1
Omentum	1	—
Lung	1	1
Peritoneum	1	1
Appendix	—	1
Tibia	—	1
<i>III. Rare gynaecological conditions</i>		
Cysts of the corpus luteum	1	1
Ectopic pregnancy	10	5
<i>IV. Miscellaneous pathological conditions</i> (including tissues from experimental animals)		
	438	279
<i>V. Tissues found to be normal on histological examination</i>		
	49	52
<i>VI. Tissues received in a state unfit for examination</i>		
	1	1
<i>VII. Brains of animals for examination of Negri bodies</i>		
	142	150
Total ..	744	651

The regional distribution of the malignant tumours is given in tables II and III.

Most of the specimens were removed during life and sent for diagnosis of the pathological condition. Wherever possible, the microscopic diagnosis of biopsy material was confirmed after operative removal of the whole specimen. Post-mortem material was received occasionally for study of the morbid histology.

Some of the interesting specimens received during the last two years are mentioned below :—

Pseudohypertrophic muscular dystrophy; chorionepithelioma; acute yellow atrophy of the liver; tuberculosis of the appendix; bilateral tubal gestation; bilateral tubal abortion; gestation in an accessory uterine cornua; Monkeberg's sclerosis of brachial and uterine arteries; filarial cyst of the knee; cast discharged from urethra containing microfilaria; bilharzial disease of the bladder; recurring basal cell carcinoma.

In addition to the above, examination of brains from suspected rabid dogs for the presence of Negri bodies was done as a routine measure. Photomicrographs of some rare and interesting conditions were prepared for illustrating case reports.

TABLE II :—Showing the regional distribution of the malignant growths received during the year 1942

	Penis	Back	Foot	Loin	Anus	Tongue	Tonsil	Calf	Breast	Arm	Forehead	Cervix	Uterus	Peritoneum	Stomach	Liver	Spleen	Vulva	Ovary	Testis	Skin	Thigh	Lymph nodes	Total
Squamous-cell carcinoma ..	3	1	3	1	1	1	1	1								1				1				14
Basal-cell carcinoma ..									1	1														2
Adenocarcinoma ..												2	2	3										7
Carcinoma simplex ..									5			2				1	1	1						10
Carcinoma papillary ..																		1						1
Seirrhoma ..																			1					1
Carcinoma secondary ..													2	1										3
Sarcoma ..		1								1												1		3
Malignant lymphoma ..																								4
Total ..	3	1	4	1	1	1	1	1	5	1	2	4	2	2	3	1	2	1	2	1	1	1	10	51

TABLE III :—Showing the regional distribution of the malignant growths received during the year 1943

	Breast	Uterus	Testis	Ovary	Cervix	Vagina	Foot	Neck	Nose	Forehead	Jaw	Ear	Skin	Scalp	Leg	Pleura	Mesentery	Toe	Lip	Abdomen	Chest	Bladder	Pelvis	Thigh	Scapula	Wrist	Inguinal region	Arm	Axilla	Epigastric region	Total
Squamous-cell carcinoma ..				8	1	1	2	1	1	1	1	1	1					1	1	1	1										21
Basal-cell carcinoma ..							1	1																						1	3
Adenocarcinoma ..	2	2																													4
Carcinoma simplex ..	6		1				1												1			1									10
Do. papillary ..			1																												1
Do. embryonal ..			1																												1
Do. secondary ..	1						2								1	2	1				1							1			9
Choriocarcinoma ..	1																											1			1
Sarcoma ..	1						1																2	1	1	1	1				8
Malignant lymphoma ..							3												1									2			6
Total ..	7	4	1	4	8	1	2	7	3	1	1	1	1	1	1	2	1	1	1	3	1	1	1	2	1	1	1	1	3	1	64

XI. Department of Virus Diseases Including Rabies

During the years under review, 7,818 persons attended for anti-rabic treatment at the Institute. Out of these, 7,009 completed the full course of treatment, 794 were advice cases and 15 discontinued treatment of their own accord. In cases where there is a doubt about the condition of the dog inflicting the bite, and the dog is available, the patient is given two injections and is advised to keep the dog under observation for 14 days. At the end of this period of observation, if the dog is healthy, no further treatment is given and the case is classified as an "advice case".

Two cases developed hydrophobia after prophylactic treatment, one in 1942 and the other in 1943. The first case, a boy of 7 years, was bitten by a dog and had deep and extensive bites on the right thigh. The treatment was started and the wounds were cauterized within 5 hours of being bitten. A full course of Class III treatment was given. The boy developed hydrophobia 7 months after the completion of the treatment. The second case was that of a woman, aged 20. She was bitten by a rabid dog on the right ankle. There were two superficial and two deep punctured wounds. The wounds were cauterized and the treatment was started within 14 hours. A Class II treatment was given. She developed hydrophobia 30 days after the completion of the treatment.

A total of 15,047 anti-rabic treatments were supplied to different centres, and of these 4,493 were issued for veterinary use.

The following new anti-rabic treatment centres were opened during the period under review, bringing the total number of outcentres to 103 :—

- (1) Civil Hospital, Narsingpore.
- (2) State Hospital, Sangli.
- (3) Civil Hospital, Vaikunthpur.
- (4) Railway Hospital, Nagpur.
- (5) Railway Hospital, Jubbulpore.

The details of treatment carried out at the Institute and the outcentres in 1942 and 1943 are summarised below

Details	1942	1943
Number of persons applied for treatment	3,582	4,236
Advice cases	367	427
Fully treated	3,211	3,798
Discontinued treatment	4	11
Percentage of health returns	98·2	Awaited.
Cases of hydrophobia	1	1
Mortality rate	0·03	0·03
Number of anti-rabic centres	103	108
Number of treatments sent to the centres	7,163	7,884
Number of treatments sent to the Veterinary Surgeons. .	465	4,028

Table showing the number of brains received and examined at the Institute for evidence of rabies during the years 1942 and 1943

Details	1942		1943	
	Number	Percentage	Number	Percentage
Positive for rabies (Negri bodies present)	104	73.2	100	66.7
Negative for rabies (Negri bodies absent)	34	24.0	46	30.7
Received in a state unfit for examination	4	2.8	4	2.6
Total	142		150	

Source of brains received and examined for rabies

	1942					1943				
	Positive	Negative	Unfit for examination	Total	Percentage	Positive	Negative	Unfit for examination	Total	Percentage
Bombay City and Suburbs	50	23	3	76	53.5	52	21	1	74	49.3
Outstations	54	11	1	66	46.5	48	25	3	76	50.7
Total	104	34	4	142		100	46	4	150	

Statement showing the classification of different animals with the results of their brain examination for rabies

Animal	1942				1943			
	Positive	Negative	Unfit for examination	Total	Positive	Negative	Unfit for examination	Total
Dog	101	30	4	135	92	42	4	138
Monkey	—	—	—	—	0	1	0	1
Goat	—	—	—	—	3	0	0	3
Fox	—	—	—	—	1	0	0	1
Buffalo	1	0	0	1	1	0	0	1
Bullock	—	—	—	—	1	0	0	1
Mah	—	—	—	—	0	2	0	2
Cat	0	1	0	1	2	1	0	3
Total	102	31	4	137	100	46	4	150

Canine rabies prophylaxis.—The President of the Margao Municipality sought help of the Haffkine Institute for control of rabies in Margao Town. He was advised to license dogs and immunise them with a single dose of 6 c.c. of a 25 per cent suspension of fixed brain virus in glycerin saline. In addition, muzzling order for dogs was to be enforced and unlicensed dogs were to be destroyed. To evaluate the results of mass prophylaxis on a statistical basis, it was suggested that the dogs of the neighbouring municipalities should be used as controls. The municipalities adjacent to Margao have agreed to extend full co-operation by adopting all preventive measures adopted by the Margao Municipality, except the administration of rabies vaccine. The Margao Municipality was supplied with 3,500 doses of vaccine in May 1943, and the data which the President of the Municipality is collecting are awaited with interest. No absolute protection is claimed for the vaccine, but it is believed that, by raising the level of the "herd" immunity the incidence of rabies would fall.

XII. Blood Bank

In collaboration with the Red Cross Society, a blood bank was organised in April 1942, to meet the existing emergency. Two centres were opened in the city and one at the Institute to receive blood donations. The Institute made itself responsible for processing all the blood donations received. With a handsome contribution from Sir Alwyn Ezra the necessary equipment was purchased and a Strumia type of plasma drying machine was built. Blood was processed into plasma and this was preserved by drying it from the frozen state. Moisture content of less than 1 per cent was consistently achieved. Throughout sulphathiazole was added to the citrate solution to get a final concentration of 0.48 per cent in the blood. Though the blood was collected and processed by the close method, it was still considered desirable to use sulphathiazole because work had to be carried out in open rooms. Sterility of each batch was tested and the addition of sulphathiazole seems to have proved very valuable in keeping down contaminations. In carrying out sterility tests, para-amino benzoic acid was added to the medium to neutralise the action of sulphathiazole. Further samples of different batches of dried plasma were frequently tested biologically by the Department of Pharmacology to determine and maintain the quality of the product.

2,981 donors in 1942 and 2,027 donors in 1943 donated blood. These donations yielded 169.75 litres and 155.84 litres of plasma in the two years. Some liquid plasma was used as such, but the major part was preserved by drying it from the frozen state.

XIII. Income and Expenditure

The following table shows the income and expenditure of the Haffkine Institute :—

Receipts		Expenditure	
	1941-42		
	Rs.		Rs.
XXVIII—Public Health—		39-D—Public Health—	
Sale proceeds of sera, vaccines		D-Bacteriological	
and miscellaneous receipts	5,85,705	Laboratories—Haff-	
		kine Institute, and 39-	
		E—Public Health—E-	
		Pasteur Institute—	
		Pay of officers ..	73,618
		Pay of establishment.	1,05,620
		Allowances, honoraria,	
		etc.	11,979
		Contingencies ..	2,65,213
Total receipts ..	5,85,705	Total expenditure ..	4,56,430

1942-43.

	Rs.		Rs.
XXVIII—Public Health—		39-D—Public Health—	
Sale proceeds of sera, vaccines		D-Bacteriological	
and miscellaneous receipts	6,75,496	Laboratories—Haff-	
		kine Institute, and 39-	
		E—Public Health—E-	
		Pasteur Institute—39-	
		G. P. H. G-5- Re-	
		search Scholarship,	
		39-G. P. H. G-7-	
		Serum Department—	
		Pay of officers ..	1,01,653
		Pay of establishment	1,19,645
		Allowances, honoraria,	
		etc.	27,597
		Contingencies ..	4,60,806
Total receipts ..	6,75,496	Total expenditure ..	7,09,701

PART II

RESEARCH WORK

1. Sulphadiazine and Sulphathiazole in the Treatment of Bubonic Plague

(SOKHEY AND WAGLE)

In November 1943, plague broke out in and around Poona. The occasion was utilised to test the comparative curative value of sulphadiazine and sulphathiazole. The Director of Public Health for the Government of Bombay, offered the required facilities at the Infectious Diseases Hospital, Poona. The field trial lasted from 15th November 1943 to 16th February 1944. During this period, 186 cases were treated, out of which 182 proved to be cases of bubonic plague.

Division of cases.—Two treatments were given in strict rotation; no selection of cases was made for a particular kind of treatment. In spite of this, the number of cases given in the tables do not work out to be equal for the two treatments. This is due to two reasons. Cases which were moribund at the time of admission and died within 24 hours were excluded, as it was found that the therapeutic concentration of the drugs was not attained till 6 to 8 hours after the administration of the drugs, and the drugs were not effective within such a short period. One case left the hospital against medical advice, while still very ill.

Diagnosis.—On admission, the diagnosis was first made on clinical grounds. At the same time, before any treatment was given, 0.5 c.c. of blood was drawn from a vein and planted in equal quantities on two agar slopes. This was done to determine the presence or absence of plague septicaemia at the commencement of treatment. If the cultures remained sterile after two days' incubation at room temperature, and there was still doubt about the diagnosis, the bubo was punctured with a syringe and the fluid cultured on agar slopes to confirm the diagnosis.

Dosage and concentration of sulphonamides in blood.—Sulphathiazole was given in the dosage of 10 g. on the first day and 6 g. on subsequent days; but as very serious cases did not respond to this dosage, it was increased in such cases to 12 to 14 g. on the first day and 6 to 9 g. on subsequent days. The concentration in the blood was estimated every morning at a fixed time. In the case of the smaller dose, the concentration in the majority of cases varied between 5 and 7 mg. per 100 ml.; in the case of the larger dose, the concentration was found to be between 8 and 12 mg. per 100 ml. The higher concentration was not found to be more effective, and the larger dose was discontinued.

Sulphadiazine was given in a dosage of 10 g. on the first day and 6 g. on subsequent days, later on, in a few very serious cases an increased dose of 14 g. on the first day and 6 to 9 g. on the subsequent days, according to the clinical condition of the patient. The former dosage gave a blood-concentration of 10 to 15 mg. per 100 ml., while the latter gave a concentration of 15 to 20 mg. per 100 ml. The higher concentration of 15 to 20 mg. per 100 ml. was not found to be more effective, and the larger dose was discontinued.

The administration of both the drugs was continued for two days after the clinical condition improved and temperature became normal. In more severe cases, the administration of the drug lasted for about ten days in all.

Results.—Out of 182 plague cases treated with sulphadiazine or sulphathiazole, twenty-five have been left out of consideration, as twenty-four were moribund cases and one left hospital against medical advice.

The results obtained in the remaining 157 cases are summarised in the following table:—

All cases of bubonic plague treated

Treatment	Number of cases	Number of deaths	Case mortality per cent
Sulphathiazole	76	16	21.05
Sulphadiazine	81	10	12.34

Since septicæmic cases almost always prove fatal, a better idea of the curative power of any treatment is gained, if only results in cases with septicæmia at the commencement of the treatment are taken into consideration. Results in septicæmic cases are, therefore, given below separately.

Cases with plague septicæmia at the commencement of treatment

Treatment	Number of cases	Number of deaths	Case mortality per cent
Sulphathiazole	39	15	38.46
Sulphadiazine	43	9	20.93

These results do not show any statistically significant difference between the two types of treatment. Sulphadiazine, however, appeared to be less toxic than sulphathiazole and its concentration in the blood could be more uniformly maintained.

It will be noted that the patients who did not have septicæmia when the treatment with these drugs was commenced (i.e., 37 in the case of sulphathiazole and 38 in the case of sulphadiazine) show very low mortality indeed (i.e., 1 in the case of each drug). This observation gives the hope that if treatment with these drugs could be started in the very early stage of the disease, almost all lives could be saved.

II. Plague Vaccine

(SOKHEY AND HABBU)

Studies into the factors which determine the immunogenic value of a plague vaccine were continued.

(a) *The relationship of the virulence of a strain to the protective power of the live vaccine made from it.*—A fully virulent strain (53 H) was made progressively avirulent by weekly subcultures incubated at 37°C. Virulence of the strain was measured from time to time as M.L.D. for a mouse. Live vaccines were made from agar growths incubated at 37°C. of selected sub-cultures. The protective dose for a mouse was

measured as the number of organisms required to protect 50 per cent of the mice against 10 times the M.L.D. (about 100 organisms) of a virulent strain. Following results were obtained :—

Number of the sub-culture	Virulence measured as M.L.D. for a mouse in number of organisms	Immunogenic value measured as mouse protective dose in number of organisms
1st	5	Indeterminable.
15th	500*	6†
25th	300,000,000	875
31st	600,000,000	3,850
36th	1,500,000,000	11,250
40th	2,000,000,000	13,500
50th	3,000,000,000	15,700
65th	Ceased to produce infection.	No protective power. 200,000,000 organisms failed to protect.

* In the Report for 1940-41, this figure was given as 25 through clerical error.

† A number of animals died from this dose before test dose could be given, test dose was given to those which survived for 14 days after the first dose of the vaccine.

It is obvious that immunogenic power of the organism diminishes as its virulence is reduced, and a point is reached where it ceases to have either invasive power or protective power.

(b) *Killed vaccines made from virulent and avirulent strains.*—Measurements were made to see if the relationship of virulence to protective power noted in the case of live avirulent vaccines, held good in the case of killed vaccines :—

Virulence as mouse M.L.D. in number of organisms	Vaccine	Mouse protective dose in number of organisms
5	Heat killed	6,500,000
300,000,000	Live vaccine	875
300,000,000	Heat killed	5,300,000

These experiments have not been completed to a logical end, but the observations so far made show that even considerable differences in the virulence of strains do not make any difference to the protective power of a heat killed vaccine. The number of killed organisms required to protect, of course, is much larger than that of live organisms.

(c) *A possible explanation of the high protective power of live vaccines.*—When it was observed that the number of live organisms (live avirulent vaccine) was very much smaller than the number of killed organisms (heat killed vaccine) required to protect a mouse, two explanations suggested themselves, either that the killing process reduced the immunogenicity of a strain or that the live organism, though highly avirulent, still retained residual virulence to enable it to multiply in the body.

In the previous report, we had reported experiments in which we had employed different means of killing cultures—such as heat, 54°C., merthiolate 1 : 500,000, sulphathiazole 1 per cent, alcohol (Felix). We worked with highly virulent organisms

and prepared vaccines from agar growths incubated at 37°C., and it did not matter with what agent we killed the organism, so long as the organism was killed, it required 6 to 7 million organisms to protect a mouse against a standard infective dose, while as few as 875 organisms of an avirulent strain were enough to protect.

To test the second hypothesis, we gave a sublethal dose (20,000,000) of a sub-culture which required 300,000,000 organisms to kill a mouse, and on sacrificing the animal 24 hours later we found that the organism, though extremely avirulent, had invaded the body and could be recovered from the spleen, showing that it had still invasive power. This is also borne out by the fact that when virulence of the organism is diminished still further as shown in paragraph (a) above, it ceases to have any protective power. It, therefore, appears that for a live avirulent organism to have protective power at all, it must have enough residual virulence to invade the body and multiply. Because of this circumstance, the use of live avirulent vaccine involves some risk. Further, under conditions in which the vaccine has to be prepared in a central laboratory and distributed over a large country, the use of a live avirulent vaccine is precluded.

(d) *Comparison of the mouse and the rat as test animals for determining the protective power of a plague vaccine.*—From what has been given above, it follows that the choice is between a killed agar grown (37°C.) vaccine or a killed broth grown (28°C.) vaccine. But before such a choice can be made, we must be clear about the relative protective powers of the two vaccines. The results, which we have reported from time to time and which showed that the protective power of the two vaccines is about equal, were obtained by using Haffkine Institute inbred white mice as the test animals. Some workers have reported that different results are obtained if white rats are used as the test animals, i.e., agar grown (37°C.) vaccine is much more potent than broth grown (28°C.) vaccine when tested on white rats. To test this finding, we have carried out parallel determinations of the protective power of the two vaccines on mice and rats. For this purpose, mice weighing between 25 g. and 30 g. and white rats weighing between 200 g. to 270 g. were used, and an infective dose of about 100 organisms was given to each mouse and about 10,000 organisms to each rat. The method of determining the minimum protective dose we have described elsewhere. The following results were obtained:—

Vaccine	Mouse protective dose	Rat protective dose
Haffkine (Broth) Vaccine, 4 weeks' growth (28°C.)	0.004 c.c.	0.030 c.c.
Agar Vaccine (37°C.), 1,000 million organisms per c.c.	0.004 c.c.	0.028 c.c.

We have made several determinations and do not find that the test animal—rat or mouse—makes any difference to the relative protective power of the two vaccines. Other things like toxicity, keeping quality, etc., being equal, either of the two vaccines could be used with the same effect.

(e) *Toxicity of broth and agar grown vaccines.*—The toxicity was measured as the dose which killed 50 per cent of the mice used—weight 26 to 27 g.

Vaccine	Toxic dose
Haffkine (Broth) Vaccine, 4 weeks' growth—28°C.	0.2 c.c.
Agar Vaccine (37°C.), 1,000 million organisms per c.c.	1.0 c.c.

We have made several such determinations and find that agar vaccine is much less toxic.

(f) *Keeping qualities of broth and agar vaccines.*—Agar and broth vaccines were made from the same strains. Agar growth was incubated at 37°C. for 3 days and suspended in saline to have a content of 1,000 million organisms per c.c. and was heat killed (54°C.) and 0·5 per cent phenol was added. Broth vaccine was a 4 weeks' growth at 28°C. heat killed and similarly carbolised. Each vaccine was divided into two portions, one was stored at 0°C. and the other at 45°C. The following results were obtained :—

Vaccine	Mouse protective dose
Broth vaccine stored at 0°C.	0·0052 c.c.
Broth vaccine stored at 45°C. for 8 weeks	0·0040 c.c.
Agar vaccine stored at 0°C.	0·0035 c.c.
Agar vaccine stored at 45°C. for 6 weeks	0·0060 c.c.

The work is still proceeding, but there is enough evidence to show that agar vaccine has poor keeping qualities. We have had the occasion to examine a batch of broth vaccine which had been lying at room temperature for about 10 years and it still shows a very high protective power.

(g) *Improvement of the broth vaccine.*—The observation that broth vaccine has superior keeping properties than agar vaccine, that it does not deteriorate when exposed to high temperature, is a consideration of extreme importance in a country in which high temperatures prevail and refrigerating facilities for storage are scarce. Broth vaccine is the vaccine of choice, if its toxicity can be reduced. Desired improvement lies in two directions ; (1) increased potency and (2) decreased toxicity.

(1) *Increased potency.*—In the case of a broth vaccine, the increase in potency largely depends on obtaining a better growth. We have shown before that 28°C. is the optimum temperature, and pH 7·2 the optimum hydrogen on concentration for the growth of *P. pestis* and have used them for preparing our broth vaccine. Further improvement in growth could only come from a change of medium. So far we have used broth made by hydrolysing mutton with HCl. We have now tried broth prepared by hydrolysing casein with H_2SO_4 and HCl—casein hydrolysate. We find that this medium gives a much better growth and, therefore, a more potent vaccine.

(2) *Reduction of toxicity.*—Formalisation readily suggested itself. We have tried the effect of formalin on our new broth vaccines with the following results :—

Vaccine	Toxic dose (dose producing death of 50 per cent of mice used)
Casein hydrolysate, 4 weeks' growth, heat killed (54°C.) and carbolised (0·5 per cent)	0·2 c.c.
Casein hydrolysate, 4 weeks' growth, 0·05 per cent formalin, with 1 mg. phenyl mercuric nitrate per 100 c.c.	0·4 c.c.
Casein hydrolysate, 4 weeks' growth, 0·075 per cent formalin, with 1 mg. phenyl mercuric nitrate per 100 c.c.,	0·6 c.c.

Preliminary results show that these amounts of formalin do not adversely affect the potency of the vaccine. Its keeping qualities are being studied. It seems we have developed a less toxic and more potent vaccine than the Haffkine Vaccine.

III. Comparative Study of Human Immunisation with Two and Three Doses of Tetanus Toxoid

(D. C. LAHIRI)

Two groups of 23 men each were immunised with the same batch of tetanus toxoid with (a) two doses of 1 ml. at an interval of six weeks, and (b) three doses of 1 ml. at intervals of three weeks. In each case, 4 weeks after the last dose, i.e., 10 weeks after the first dose, samples of blood were drawn and titrated for their antitoxin content. Thus, though the period of immunisation in both cases was the same, the group immunised with three doses showed, on the average, much higher antitoxin content than the group immunised with two doses. This experiment shows that tetanus toxoid should be used in three doses and not in two.

IV. Keeping Qualities of Tetanus Toxoid preserved with Phenol, Phenyl mercuric nitrate and without a Preservative.

(D. C. LAHIRI)

A batch of tetanus toxoid was divided into three equal portions. One of them was preserved with 0.5 per cent phenol, the second with 0.002 per cent phenyl mercuric nitrate and to the third no preservative was added. Each portion was then divided into two equal parts. One part of each portion was stored at 37°C. and the other at 0°C. The mouse protection values of each of these parts of toxoid were determined from time to time. The results showed that at 37°C., the portion preserved with phenol showed deterioration of the protective power much earlier than the portion preserved with phenyl mercuric nitrate or the portion without a preservative. No loss of potency was observed in either of the samples when stored at 0°C. for over six months. These experiments indicate that phenol should not be used as a preservative in tetanus toxoid. It is best not to use a preservative at all, but if one is used, phenyl mercuric nitrate is better than phenol. Tetanus toxoid should better be left unpreserved; tetanus toxoid shows mild bacteriostatic action in itself. If the doses are dispensed in ampoules in such a way that the whole amount is used at once, there is no great risk of contamination of the toxoid during administration.

V. Keeping Qualities of Tetanus Antitoxin dried from the Frozen State

(D. C. LAHIRI)

Even when liquid antitoxins are stored in refrigerators (0°-5°C.), they gradually lose their potency. The loss of potency is much more rapid when stored at room temperature. In India, where the prevailing room temperatures are high and refrigeration facilities almost non-existent in villages and small towns, the problem of storage of these biological products is a serious matter. With the availability of processes for preserving biological products by drying them from the frozen state, the problem can be solved readily. With this object in view, the keeping qualities of tetanus antitoxin dried from the frozen state were investigated.

A batch of the concentrated antitoxin containing 2,000 I. U. per ml. was dried from the frozen state by "Cryochem Process". They were divided into 4 batches and stored at 45°C., 37°C., room temperature varying from 28° to 32°C. and 0°C. A portion of the same batch of the antitoxin was also dispensed in liquid form in

ampoules, and stored under the same conditions as above. The antitoxin titres of the samples were determined from time to time. The results were as follows :—

(1) At 45° C., the liquid antitoxin had lost more than 50 per cent of its potency (i.e., it contained less than 1,000 I. U. per ml.) in 1½ months and had jellified in two months, while the dried antitoxin showed scarcely any loss of unitage even after as long an exposure as 9 months, actual measurement being 1,900 I. U.

(2) At 37°C., the liquid antitoxin had lost more than 50 per cent of its potency (i.e., it contained less than 1,000 I. U. per ml.) in 12 months, but had not yet jellified, while the dried antitoxin, even after an exposure to this temperature of 22 months, showed no loss of potency. The dried antitoxin dissolved rapidly and formed a clear solution.

(3) At room temperature, the potency of the liquid antitoxin had fallen from 2,000 I. U. to 1,400 I. U. after 12 months and to 1,200 I. U. after 22 months' storage. Because of the absence of loss of potency after storage at 37°C., the dried samples stored at room temperature and at 0°C., have not been tested yet.

VI. On the Ecology of Plague

(SHARIF AND NARASIMHAM)

Thirteen villages in the Dharwar District and three villages in the Belgaum District were surveyed with a view to detect plague infection both in the domestic and wild rodents from January 1942 to March 1943. In addition to the trapping carried out by the Municipality, different sections of Dharwar Town were surveyed systematically. A few rodents and their ectoparasites from Hubli Town were also examined. Villages Narendra, Mugad and Alnavar and Malapur section in Dharwar Town suffered from plague at the time of survey. Mugad, Alnavar and Malapur were definite instances of carry-over of plague from one season to another.

In all 24,211 domestic rodents and 1,301 wild rodents were examined. Plague infection was detected in one house-mouse (*Mus dubius*) and two brown bellied domestic rats (*Rattus rattus rufescens*) from Narendra, and three white bellied domestic rats (*Rattus rattus wroughthoni*) and twelve brown bellied domestic rats from Alnavar. No infection was detected in the wild rodents or their ectoparasites.

The ectoparasite-inoculation tests as usual proved more useful in detecting and determining the duration of plague infection than the examination of rodents. They provided a definite proof for carry-over of plague from one plague season to another in the village of Alnavar and the manner in which it was effected. In the case of domestic rodents, 853 ectoparasite and tissue-inoculation tests were carried out, and out of which 36 ectoparasite and five tissue-inoculation tests were positive to plague. All the positive tests except one with ectoparasites collected from Lingannath were from the material obtained from Alnavar. The former village which is about two miles away from Alnavar reported no human case before or after the detection of plague in the ectoparasites. Only 58 inoculation tests with tissues and ectoparasites of wild rodents were done, and they all turned out negative to plague. Of the 145 culture tests carried out, 30 were positive to plague. Infection was once detected in a tick and a flea collected off *Suncus caruleus* from village Narendra.

During the period of survey, in addition to other ectoparasites, more than 1,57,618 fleas from domestic rodents and 8,509 from wild rodents were collected, and out of these more than 1,02,033 and 5,000 respectively were identified. The

fest were utilized along with other ectoparasites for inoculation tests. Two apparently new species of lice were found on domestic rats and bandicoots from the localities situated in the hilly tracts. A very rare flea *Acröpsylla episema* Rothschild, hitherto described from one female from Dacca was found on a musk-shrew and rats of Dharwar Town and village Kakeri. The description of the hitherto unknown male will be published soon. *X. cheopis* is essentially a flea of domestic rats and *X. astia* that of *Tatera Indica* and *Bandicota malabarica* in this area. The latter flea was also found in small proportions on the domestic rats of premises in the proximity of fields or of those infested with bandicoots. *X. brasiliensis* was also found in large numbers on domestic rats of localities situated in the hilly and woody tracts.

Constant differences in the proportions of the different species of fleas in the various sections of Dharwar Town necessitated its extensive and systematic survey, which yielded interesting results. It was observed that one section of the town did not contain *X. brasiliensis* at all, whilst the other had this species to an extent of over 50 per cent of the entire flea population. Its gradual increase from one section to another was also noticed. Moreover, wherever it was present, it was found that certain houses had exclusively *X. brasiliensis* and others solely *X. cheopis*. The former species predominated in cool, damp and ill-ventilated houses, and the latter in the comparatively dry and well-ventilated houses.

In Dharwar Town, it was observed that moderately clean and pucca houses of the bungalow type harboured very few rats and comparatively more mice were trapped in them than rats. Heavy infestation of domestic rats was noticed in the houses of farmers containing grain storage and cattle.

The percentage of prevalence of white bellied domestic rats in different localities was very variable. It was highest in the villages situated on hard hilly ground. Similarly, in Dharwar Town, it was very high in sections situated on hard ground. It was very low in localities situated on soft ground. *Gunomys kok* was exclusively a wild rodent in the Dharwar District and was very rarely trapped from residential premises. *B. malabarica* was a domestic rodent and was very common in localities situated on soft ground. *Suncus caruleus* was caught comparatively in larger proportions from infected villages, when the epizootic was either in full swing or had recently subsided.

One interesting feature noticed about the domestic rats in Dharwar and Barsi talukas was that comparatively large numbers of rats were trapped during the rainy seasons. It is reasonably suggested that large number of rats that usually live in rubbish heaps, stakes and other shelters in the court-yards or round about the houses come within doors during the rainy season, and consequently, there is a sudden increase in rat population inside the rooms.

The findings of the Plague Recrudescence Inquiry in Sholapur and adjoining districts do not support the idea that the wild rodents help to carry over the infection from one plague season to another. In this area, wild rodents cannot be considered to be responsible for plague, though they have been shown to be so in Transbaikalia, Mongolia, South-Eastern Russia, South Africa and the western parts of the United States of America. In this Province, the domestic rats only perpetuate the infection. In suitable places, the infection among domestic rats goes on throughout the year. The infection is not apparent in the hot season, because its intensity is diminished and it runs a course of slow subterranean enzootic from burrow to burrow. The end of the off-season is marked by the advent of the rainy season which operates in two ways. Firstly, it causes the rats from outside shelters to herd into burrows within doors and remain there perforce, which

results in a considerable increase in rat population within houses. Secondly, it lowers the temperature and raises the humidity to such an extent as to result in a striking rise in the flea population and to permit fleas to come out of burrows to attack human beings. A stage is thus set for infection to flare up.

The study of the state of infection in the areas surveyed has led to the conclusion that there are two distinct types of plague epizootics. In most of the villages in the comparatively warm and low tablelands, the infection is short-lived and flares up intensively, resulting in explosive epizootics amongst rats and getting more or less completely extinguished within a few months. During the summer, the infection often disappears from most villages, so much so, that appearance of plague in them is more or less a result of fresh importation from commercial centres, which are capable of retaining the infection for a much longer time than the small villages, on account of large population of domestic rats and comparatively lower temperature in the burrows in the grain godowns. This is apparently due to a greater multiplication of fleas and plague organisms in them during the cooler months and a severe check on their activities during the hot and dry months of summer. On the other hand, in the cooler villages with marked infestation of *X. brasiliensis* lying in the hilly and woody tracts on the watersheds of the Western Ghats, especially in the Belgaum and Dharwar districts the infection is slow spreading and lasts much longer due to the retarding effect of the lower prevailing temperature during the comparatively colder winter on the increase of fleas and organisms in them. Consequently, it does not lead to a very high and quick rate of mortality amongst the rodents. The reoccurrence of plague in some villages in these areas is the result of carry-over. The climatic conditions of these cooler regions approximate to what they should be in endemic centres, as the factors here are very favourable for lingering on and carry-over of plague infection from one season to another.

VII. Plague endemic Centres in India, and Spread of Plague in the Southern Registration Districts of the Bombay Province

(SHARIF)

The analysis of about seven years' epidemics in the Southern Registration Districts of the Bombay Province has revealed that plague in the Belgaum, Dharwar and North Kanara districts radiates from the eastern watersheds of the Western Ghats. Ever since its commencement the plague has never left this area. It lingers on here for much longer duration than elsewhere and its progress is comparatively slow.

From the past records of plague in India, it appears that plague has clung since long to cool and comparatively damp areas comprising of watersheds situated in the submontane regions and high tablelands varying in altitudes from 2,000 to 4,000 feet. They can conveniently be designated as endemic centres. Plague, being a migratory disease, moves from place to place even in these endemic centres. It is never confined to any one definite locality for long, though it persists in certain adjacent areas having more or less identical climatic conditions. Three such endemic centres are located in the submontane regions of the Himalayas of which two in the United Provinces and Bihar are still active. There is one or perhaps more in the watersheds of Central Indian Mountain Ranges. In Southern India there are three endemic centres: first one in His Exalted Highness the Nizam's Dominion comprising of watersheds of the Balaghat Range and the adjoining hills; the second one containing eastern watersheds of the Western Ghats in the Dharwar and Belgaum districts and the Mysore State, the third one including watersheds of Shevaroy Nilgiri, Palni and Cardamom hills. Plague radiates from

These endemic centres and the limits of the infected areas increase or decrease very gradually, showing a secular tendency. The extent of the area invaded by plague depends upon the intensity with which it spreads and the momentum it attains in its march; on reaching the warmer lowlands, it loses its vigour and finally disappears.

Infection in Sholapur and Bijapur districts appears to be always imported from an endemic centre located in H. E. H. the Nizam's Dominion. Both the Sholapur and Bijapur districts were free from plague in the years 1937 and 1938. Plague appeared in March 1939 first in the Barsi Taluka and by September 1939 it had moved southwards into the Sholapur Taluka. If the plague infected villages in these two talukas of the Sholapur District and talukas of the Bijapur District are arranged according to the date of commencement of plague incidence, it becomes quite apparent that plague moves from H. E. H. the Nizam's Dominion first towards the Barsi Taluka then towards the Sholapur Taluka and finally into the Bijapur District.

It has been observed that plague moves along the trade routes. Commercial centres get infected first and then plague radiates from them into the neighbouring villages. In the non-endemic area, such as Sholapur and Bijapur districts first a few localities get infected in one plague season and then a very large number of them in the following plague season. In the third plague season, only a few of them suffer and then plague disappears for about two years. The sudden flare up of human plague in the second effective plague season in some villages tends to show that dissemination of plague can take place through rat-favoured merchandize even during the off-season. In these districts the mass movement of agricultural commodities starts after the harvesting season and is in full swing before the monsoon starts; then it is considerably reduced with the onset of rains. The rains in July increase the intensity of epizootic and human cases start in August, which continue increasing up to the end of September. From October to middle of November, due to behaviour of adult fleas towards a rise in temperature, there is a decline in human cases. From December to February there is a gradual increase in human plague. The decline starts in March and plague stops with the onset of the off-season in most villages.

VIII. The Nutritional Requirements of the Larvae of the three common Indian Species of *Xenopsylla*, and their bearing on specific Distribution

(SHARIF)

Previous studies on the nutritional requirements of the larvae of the three common Indian rat-fleas lend strong support to the conclusion that complete development of the flea larvae is unobtainable unless certain accessory food substances, such as vitamins of the B-group, are included in their diet. There is enough indirect evidence to show that the source of these vitamins in nature under certain conditions is the association of micro-organisms especially the fungi with the food. Excessive increase in the flea population is always due to presence of micro-organisms in the larval diet. Under normal dry conditions the sources of vitamins are different agricultural products that are either found in undigested form in the faeces of hosts or intact in their burrows.

It is reasonably suggested that irregular and patchy distribution of the three common rat-fleas in India and their host preferences are governed by the nesting conditions and the nature of the food of their hosts in combination with the climatic conditions of the place. The dietetic requirements of the larvae of the three common Indian rat-fleas vary according to the species.

The larva of *X. astia* requires a more nutritive diet than those of the other two species and its temperature tolerance is also higher. This species flourishes well in the burrows of *Tatera indica* (Hardwick) and *Bandicota malabarica* Shaw, where a rich food for the flea larvae is available. This species cannot flourish in the domestic rat burrows in the Sholapur and Dharwar districts, in which nutritive value of the nesting conditions is very poor. Wherever the débris and litter rich in vitamins and proteins are present in the burrow of a rodent either as the result of putrefaction or due to the presence of substances rich in nutritive value and the burrow temperature can rise beyond 36° C., *X. astia* is sure to be the sole flea.

Nutritional requirements of the larva of *X. cheopis* are comparatively poor, and it can easily breed in burrows containing food poor in nutritive value. The temperature tolerance of the larva of *X. cheopis* is not so high as that of *X. astia*. The former can prosper readily in the burrows of the domestic rat in most areas, which provide a poor food for flea larvae. Consequently, it is the flea of this rat in many parts of the world. There is a very close association between grain storage on one hand and *R. rattus* and *X. cheopis* on the other hand. This explains the great part played by grain trade in the dissemination of plague.

On the other hand, nutritional requirements of the larva of *X. brasiliensis* are of a different nature and it thrives better on cereal proteins than those of the other two species. The temperature tolerance of this species is the lowest of all the three species. Consequently, this flea is only found in the cooler regions of the Peninsular India. It was present in the hilly and woody areas situated in the Dharwar Taluka and notably absent on the domestic rats in the villages located in flat barren areas. It was found that a village in flat barren country even when only about a mile away from the one in the hilly forest region did not include it in its fauna, whilst it was prevalent in the latter.

An attempt has been made to explain the irregular and patchy distribution of the three species in India on the basis of the relative nutritive value of the varied organic substances found in the domestic rat burrow at different places along with the range of temperature tolerance for the larvae of these species, and it has yielded very interesting results which will soon be published.

IX. On the Structure of the so-called "Penis" of the oriental Cat-Flea, *Ctenocephalides felis* subsp. *orientis* (Jordan), and Homologies of the external Male Genitalia in Siphonaptera

(SHARIF)

The anatomy and homologies of the external male genitalia of the oriental cat-flea are revised in the light of our existing knowledge of the insect male genitalia.

The abdomen of the adult flea consists of ten segments, and the last one may contain elements of the eleventh segment. The complicated structure, forming the so-called "penis", is the copulatory apparatus, as it includes the harpagones in addition to the intromittent organs. It is composed of the periphallic and phallic organs.

On account of the extension of the copulatory apparatus far into the abdomen and far beyond the level of anus posteriorly, both the ninth and tenth abdominal sterna are deeply inflected. Consequently, each of them is differentiated into a genital portion associated with the copulatory apparatus, and an anal portion in the case of the tenth sternum and a somatic portion of the ninth sternum. The V-shaped vertical portion of the so-called "boomerang shaped" sternum is the ninth abdominal sternite and the horizontal arm is its apodeme. The genital

portion of the ninth sternum is a coxosternite consisting of the posterior portion of the ninth sternum, coxal plates and harpagones forming the gonopods, and an elongated rod-like apodeme of the pons coxalis. The biggest sclerite of the copulatory apparatus is composed of the genital portion of the tenth sternum and its greatly enlarged apodeme.

The phallic organs consist of a short median aedeagus, two elongated copulatory rods possibly representing the parameres, and a phallosome. The phallosome is a permanent irreversible tubular sheath, which completely invests the aedeagus and the copulatory rods. Its posterior narrow portion is strongly sclerotized and forms a sclerotic complex consisting of the posterior sclerotic tube, the pumping apparatus, the Y-shaped dorsal sclerite and the leaf-shaped ventral sclerite. As the aedeagus, being short, cannot protrude through the external opening of the phallosome, and thus cannot act as an intromittent organ, the spermatid fluid discharged into the phallosomal chamber, is drawn up by the pumping bulb on its dilatation through an elongated aperture guarded by an inlet valve; but on the contraction of the pumping bulb the spermatid fluid is pumped out from the distal opening of the tube of the pumping apparatus in a powerful jet which is directed by the copulatory rods. The latter act as the real organs of intromission into the bursa copulatrix of the female.

The male genitalia especially the copulatory apparatus is highly muscled and a detailed account of its musculature is given.

X. Manufacture of Sulphathiazole

(GANAPATHI, SHIRSAT AND DELIWALA)

When an aqueous solution of 2-aminothiazole is treated with acetylsulphanilic chloride even in equimolecular quantities, the product obtained was found to be one in which one molecule of the aminothiazole had condensed with two molecules of the sulphonylchloride. This new dicondensation product on boiling with 10 per cent alkali or hydrochloric acid furnished sulphathiazole in good yields. This observation forms the basis for the new process of manufacture of sulphathiazole, in which an aqueous solution of 2-aminothiazole is condensed at room temperature with a slurry of two molecular equivalents of the sulphonylchloride using calcium carbonate as the condensing agent. The condensation product is obtained in good yields. This, on boiling with about 4-5 N hydrochloric acid, undergoes hydrolysis and gives sulphathiazole in good yields. The great advantage of this method is that the aminothiazole used need not at all be isolated; in practice, the solution of aminothiazole hydrochloride obtained as shown in the succeeding abstract after steam distilling is neutralised with calcium carbonate and used as such after estimating the aminothiazole concentration in an aliquot portion. The sulphonylchloride can be used straightway after washing with water free from acid by decantation. Thus, the whole process is much simplified and the working time considerably shortened.

XI. Preparation of 2-Aminothiazole and 2-Amino-5-alkyl-thiazoles

(GANAPATHI, SHIRSAT AND DELIWALA)

The conditions for the large-scale preparation of 2-aminothiazole in good yields by the condensation of dichloroether and thiourea have been established. The best method for the preparation of dichloroether by the chlorination of ether has been worked out. Though all the processes could be conducted in very good yields, this method has been abandoned in favour of the other one because of the possibility of fire hazards in the chlorination of ether and the fractionation of

the chlorinated product leading to terrible corrosion of the equipment. The method which is now being used with great deal of advantage consists in chlorinating ethyl alcohol under a set of conditions, allowing the product to separate into two layers and condensing the heavier layer with an aqueous solution of the appropriate amount of thiourea. From the aqueous solution, the 2-aminothiazole is isolated in good yields. This method is very simple, very rapid and involves no distillation. The aqueous solution of 2-aminothiazole hydrochloride obtained as indicated above is used directly for the manufacture of sulphathiazole after steam distilling off the uncondensed products and neutralising. This method has been extended for the preparation of 2-amino-5-alkylthiazoles. Suitable aliphatic alcohols are chlorinated to the required extent, the resulting products condensed with thiourea and the aminothiazoles isolated from the aqueous phase by the usual processes. Thus starting from *n*-propyl, *n*-butyl, *n*-amyl, isoamyl, *n*-hexyl, *n*-heptyl alcohols and adopting the above procedure, 5-methyl, 5-ethyl, 5-*n* propyl, 5-*iso* propyl, 5-*n* butyl, and 5-*n*-amyl derivatives of 2-aminothiazole have been obtained.

XII. Synthesis of 5-Sulphanilamidothiazole and other Thiazole Derivatives.

(GANAPATHI AND MRS. VENKATARAMAN)

Though 2-sulphanilamido derivatives of thiazole have been well studied, the isomeric 4-and 5-sulphanilamidothiazoles are not at all known. Attempts were made to prepare a series of compounds of both the groups. The problem became more complicated, because the starting 5-amino and 4-amino thiazoles and derivatives were not at all known. So a number of attempts were made to synthesise them. After many trials, the optimum condition for the conversion of an ester into the amino group in the thiazole derivative was established. Thus ethyl 2:4-dimethylthiazole-5-carboxylate was converted into 2:4-dimethyl-5-aminothiazole which, on condensation with acetsulphanilylchloride, yielded 2:4-dimethyl-5-acetsulphanilamidothiazole. This, on hydrolysis, yielded 2:4-dimethyl-5-sulphanilamidothiazole, the first member of the series. Similarly, starting from ethyl 4-methylthiazole-5-carboxylate was obtained 4-methyl-5-aminothiazole, isolated as the acetyl derivative. This also yielded the sulphanilamido derivative. Other thiazole esters are being subjected to this reaction.

Another method was adopted to prepare this type of compound, but this is of limited applicability. 2-Hydroxy-4-methylthiazole condensed with diazotised *p*-toluidine to yield the expected dye. This, on reduction, yielded 2-hydroxy-4-methyl-5-aminothiazole which underwent condensation with acetsulphanilylchloride to yield 2-hydroxy-4-methyl-5-acetsulphanilamidothiazole.

Attempts to obtain acetsulphanilamidothiazole derivatives by condensing acetsulphanilylazide with thiazole, 2:4-dimethylthiazole and 2-hydroxy-4-methylthiazole according to the method of Curtius were unsuccessful. 2:4-Dimethyl-5-bromothiazole did not condense with acetsulphanilamide in the presence of potassium carbonate and copper powder, potassium phthalimide, urotropin, etc. Attempts to convert the oxime of 2:4-dimethyl-5-acetylthiazole into 2:4-Dimethyl-5-acetaminothiazole by the Beckman transformation were not successful. The best conditions for the reduction of 2:4-dimethyl-5-nitrothiazole are being established.

Some derivatives in which the thiazole ring is attached to the sulphonamido radical of sulphanilamide through methylene groups, were prepared by condensing sulphanilamide and acetsulphanilamide with 2-methylthiazole, 2:4-dimethylthiazole and 2-hydroxy-4-methylthiazole individually in the presence of paraformaldehyde and zinc chloride.

XIII. Synthesis of Derivatives of Diphenylsulphone

(GANAPATHI AND MRS. VENKATARAMAN)

Since three derivatives of 4:4'-diaminodiphenylsulphone, [viz., promine, di (formaldehyde sulfoxylate) derivative and the phosphorylated product] have been reported to show definite protective action against experimental tuberculosis in guinea-pigs, an attempt is being made to synthesise and study systematically a number of derivatives of 4-aminodiphenylsulphone with a variety of substituents in the other benzene ring in the ortho, meta and para positions. These compounds are also expected to provide additional data in extending the Bell Roblin theory of action of the sulphanilamides to the sulphones. In the first instance starting from 4-nitro-4'-aminodiphenylsulphone by Sandmeyer's reaction were obtained 4-chloro, bromo, iodo, and hydroxy compounds and 4-nitrodiphenylsulphone. The cyano compound could be prepared only by the fusion of the halogeno derivative with cuprous cyanide. These compounds, on reduction yielded the 4-aminodiphenyl derivatives. The diazotisation of 4-nitro-4'-aminodiphenylsulphide was not smooth. The condensations of 4-nitro-4'-aminodiphenylsulphone and 4-nitro-4'-aminodiphenylsulphide with picoline, 2:4-dimethylthiazole, and 2-hydroxy-4-methylthiazole were carried in the presence of paraformaldehyde and zinc chloride.

XIV. Synthesis of 2-hydroxythiazole Derivatives

(GANAPATHI AND MRS. VENKATARAMAN)

In connection with some other work, 2-hydroxythiazole and its derivatives were required. For the synthesis of these, there are two methods possible, viz. (1) the conversion of the halogenoketone into the thiocyanoketone with the subsequent isomerisation and (2) the condensation of the halogenoketone with ammoniumthiocarbamate. The merits of these two methods were compared in many typical cases. Chloroacetal or dichloroether could not so far successfully be converted into 2-hydroxythiazole. 2-Hydroxy-4-methylthiazole is best prepared from chloroacetone by condensation with ammoniumthiocarbamate; though thiocyanacetone is obtained in good yields the isomerisation into 2-hydroxy-4-methylthiazole does not proceed in good yields, a by-product being also produced. In the case of α -chloromethylethylketone condensation with ammonium thiocarbamate furnishes the 2-hydroxy-4:5-dimethylthiazole in about 40 per cent yield, this being not superior to the other method. Ammonium thiocarbamate and dichloroacetone yielded 2-hydroxy-4-chloromethylthiazole along with a resinous product; while the reaction with thiocyanate yielded as the main product 4-thiocyanomethyl-2-hydroxythiazole. α -Chloroacetoacetate with the thiocyanate yielded 2-hydroxy-5-methylthiazole-5-carboxylate, m.p. 128°, while ammonium thiocarbamate furnished in quantitative yields an isomer, m.p., 176°, which is probably a dimeric product.

XV. Treatment of Wounds with special Reference to War-Wounds.

(DIKSHIT AND GARDHAM)

This work was done in collaboration with Col. A. J. Gardham, Consulting Surgeon, Eastern Command, India. Wounds are very susceptible to infection in jungle warfare and even minor scratches and abrasions develop into a jungle sore. Experiments were, therefore, designed to see whether such infections could be prevented. Experimental work on dogs and rabbits showed that application of sulphonamides and cod-liver oil worked up into "water-in-oil" type of paste can

prevent experimental wounds getting heavily infected, even though they are smeared with infective organisms like *B. haemolytic streptococcus*, *Staphylococcus aureus* and *B. pyocyaneus*. Dressing these wounds with a cotton-wool pad impregnated with sulphanilamide helped the action of the paste. By making superficial wounds in rabbits and smearing the healthy skin around with pyogenic organisms, it was further shown that the paste-pad technique could successfully prevent the infection from travelling to the wound from the skin. The effect of application of the paste to body tissues like the voluntary muscles and cavities like peritoneum and knee joint showed that the paste could safely be applied if the wound is an open wound and that its introduction into the peritoneal or knee joint cavities should be avoided. The absorption of the sulphonamides from local application of the paste to the wound showed that the highest concentration reached in the blood was of the order of about 2 mg. per 100 ml. The drug could be detected at the site of application even after 144 hours. Comparative studies with "water-in-oil" and "oil-in-water" types of pastes showed that the absorption of sulphonamide drugs from the "water-in-oil" type of paste is much less rapid.

XVI. Formalin Sulphanilamide in Intestinal Infections

(MAHAL)

The important drawback of sulphanilamide and similar readily absorbed drugs of this group is that they do not remain in the intestinal canal for a sufficiently long time to exert their local bacteriostatic action. Attempts were, therefore, made to synthesize a drug which would not be readily absorbed and thus remain in the intestinal canal for a sufficiently long time to produce whatever bacteriostatic action it can. A combination of formalin and sulphanilamide was, therefore, prepared and was found to be non-toxic and not readily absorbed. Large doses of this drug (0.5 g.) were administered to monkeys 3 times a day for 7 days. The bacterial count (*B. coli*) of the faeces was taken for a week before commencing the treatment, during the treatment and one week after the treatment. Similar doses of sulphaguanidine were given to another series of monkeys and the bacterial counts were made in a similar way. It was found that both these drugs reduced the *B. coli* count to a considerable extent, reduction of the number of bacilli commencing about 4 days after administration of the drugs. As soon as the drug was stopped, *B. coli* count came up to normal within 2 days in case of sulphaguanidine and it required about 4 days in monkeys treated with formalin sulphanilamide for the count to come up to normal.

It was observed in case of both these drugs that although *B. coli* were reduced in number, Enterococci were not affected in any way.

XVII. Action of Ions on the Central Nervous System

(PATEL)

The action of K, Ca, Mg and NH_4 ions was studied by introducing small quantities of their chlorides into the cisterna magna of anaesthetised cats and dogs. Blood pressure, spleen volume and intestinal contractions were recorded in the usual manner. It was found that K and Ca exerted a powerful action on the vasomotor centres of the central nervous system, K acting as a stimulant and Ca as a depressant. The marked stimulant action of K could be quickly neutralized by application of Ca to the nerve centres.

A buffered phosphate solution of K prepared in the manner described by Lena Stern produced the typical effects of K and the action was much prolonged.

XVIII. Trichlorethylene as a general Anaesthetic

(DIKSHIT AND KULKARNI)

The action of trichlorethylene as a general anaesthetic was investigated and compared with that of chloroform. The new anaesthetic carried the animals through all the stages of surgical anaesthesia as did chloroform, the only important difference being that the relaxation of muscles was less complete in case of trichlorethylene as compared with chloroform. The action of the two drugs on the cardio-vascular, gastro-intestinal and genito-urinary systems was about the same. Trichlorethylene was found to precipitate cardiac irregularities in the same way as did chloroform. The action of trichlorethylene on the respiratory centre was, however, found to be one of stimulation, while chloroform produced a depression. An important difference in favour of trichlorethylene was that while prolonged chloroform anaesthesia damaged the liver to a considerable extent, this toxic action on the liver was not seen in trichlorethylene anaesthesia of comparable duration.

XIX. Thiazole Derivatives of Sulphonamide in Monkey Malaria

(PATEL)

The action of two new thiazole derivatives of sulphanilamide, (i) N¹-methylsulphathiazole and (ii) 2-N¹-sulphanilamido-5-isopropylthiazole prepared and supplied by the Department of Chemotherapy was studied in *Rhesus* monkeys infected with K₂ strain of *Plasmodium knowlesi*. Parasites in the peripheral blood were enumerated daily and when they had reached a level of approximately 100 parasites per 10,000 normal R.B.Cs., the drug was administered orally in the form of a tragacanth suspension.

Drug No. I was administered in doses varying from 1 g. given only once to 1 g. twice a day for 2½ days. The drug caused disappearance of parasites from the peripheral blood with a total dose of 2 g., but relapse occurred; smaller dose could not cause the disappearance of parasites from the blood stream. Concentrations of the drug in the blood of the monkey receiving a dose of 1 g. only once were determined. Maximum concentration reached was 0.89 mg. per 100 ml. in 7 hours, which came down to 0.42 mg. per 100 ml. in 24 hours. The percentage of conjugation in 24 hours was about 60.

Drug No. II was administered in doses varying from 0.5 g. given only once to 1.0 g. given twice a day for 2½ days. It was found that even a dose of 0.5 g. given once only caused the disappearance of parasites from the peripheral blood. No relapses occurred in the treated monkeys. The question of radical cure was, therefore, investigated in the case of animals treated with this drug. The blood of treated monkeys was found to be non-infective three weeks after the disappearance of the parasites from the peripheral blood, and the animals so treated were susceptible to fresh infection, the progress of which was normal, showing thereby that there was no residual immunity due to previous infection. Treated monkeys did not develop the infection even after splenotomy showing that the drug produced a radical cure in monkeys. Absorption of the drug in monkeys was also studied. A dose of 0.5 g. given once only produced a maximum concentration of 2.2 mg. per 100 ml. in 4 hours, which came down to 1.16 mg. per 100 ml. in 7 hours and to 0.5 mg. per 100 ml. in 24 hours. The degree of conjugation in the case of this drug was much lower.

XX. A new Method for Estimation of volatile Trihalogen Anæsthetics in the Blood and Tissues

(KULKARNI)

In this method, the Fujiwara reaction (chloroform in presence of an alkali gives a pink colour with pyridine) was utilized to estimate trichlorethylene and chloroform concentrations in blood and tissues of experimental animals anæsthetized with these anæsthetics. The material was dissolved or suspended in water and air distilled, first without application of heat and later by gently heating to about 80°C. The liberated anæsthetic was taken up in cold pyridine and estimated by comparing the colour of pyridine produced by Fujiwara reaction, with standard colours produced in the same way.

XXI. Changes in the Protein Fractions of the Plasma of Horses undergoing Hyper-immunization against Tetanus and their Relation to the antitoxic Titre

(S. K. GOKHALE AND D. C. LAHIRI)

This study was undertaken to find out whether there is any correlation between the amounts of the different protein fractions, viz. euglobulin, pseudo-globulin and albumin, and the antitoxic titre of the blood plasma of horses undergoing hyper-immunization against tetanus toxin. Very meagre data on this subject are available, although it is known in general that on immunization there is an increase in the globulin fraction and a decrease in the albumin fraction. Most of the work that has been done so far is on immunization against toxins other than tetanus.

Twenty-two horses undergoing hyper-immunization against tetanus toxin were studied for a period of about seven months. The plasma protein fractions, and the antitoxic titre were estimated in the blood of each horse at intervals of every two weeks. The protein fractions were estimated according to the method of Howe *J. Biol. Chem.*, 49, p. 109 (1921); Hawk and Bergeim, *Practical Physiological Chemistry*, 11th edition, p. 453 (1938), and the antitoxic titre was estimated by the animal inoculation method. Haffkine Institute bred white mice were used. The results obtained are summarised below.

It was found that the increase in the amount of euglobulin and the pseudo-globulin was in proportion to the rise in the antitoxic titre. The average amount of euglobulin, pseudo-globulin and albumin, which was 0.28 g., 3.12 g. and 3.22 g. respectively per 100 ml. of plasma before immunization, changed to 0.55 g., 5.71 g. and 2.06 g. per 100 ml., at the period of the highest level of immunization obtained. The bloods of two horses used as controls did not show similar changes. It may be mentioned here that the level of immunity obtained was only 1,500 International Units per c.c. of plasma, as at about the twenty-fifth week, an epidemic of surra broke out amongst the horses and the immunization had to be abandoned.

XXII. Effect of Sulphanilamide Derivatives in experimental Pneumococcal and Streptococcal Infections in Mice

(R. S. RAO, SAVOOR AND GANAPATHI)

Thirty-two new hetero-cyclic derivatives of sulphanilamide were tested in experimental pneumococcal and streptococcal infections in mice using the technique previously reported by Sanjiva Rao and Ganapathi. In preliminary studies many of these were found to show considerable activity.

XXIII. Sulphathiazole as an Agent for lowering Bacterial Content of Vaccine Lymph

(R. S. RAO)

Glycerine is the vehicle universally used in the preparation of vaccine lymph. However, while glycerine does reduce the bacterial content of vaccine lymph, it is also toxic to the virus. An ideal vehicle would be that which, while rendering vaccine lymph sterile, would exert no toxic action on the virus. Experiments conducted in the Institute had shown that sulphathiazole had no effect on the virus. A few preliminary experiments were conducted to assess the value of sulphathiazole as a bactericidal agent for vaccine lymph. Crude lymph obtained through the courtesy of Vaccine Institute, Belgaum, was ground in 0.004 M Citric acid and sodium phosphate buffer solution to obtain 1 : 6 dilutions and to this the sodium salt of sulphathiazole was added in different concentrations. The bactericidal action of each concentration was tested both at room temperature (25°-30°C.) and in the cold (0°-2°C.). There was no difference noted between the two. The potency of the batches kept in cold was tested on rabbits after varying intervals of time. The experiments showed that finer the grinding of the material the more consistent was the action of sulphathiazole. In the experiments carried out, the crude lymph could not be ground to the desired fineness for want of a proper apparatus. The following results were obtained:—

(1) A 10 per cent concentration of sulphathiazole sterilized the lymph in 24 hours. The potency of the lymph, however, dropped appreciably in 2 months' time. (2) A 5 per cent concentration sterilized the lymph in 10 days, while the potency was appreciably affected in 2 months. (3) A 1 per cent concentration sterilized the lymph in one month, while the potency remained unaffected when tested after 4 months. (4) A 0.2 per cent concentration showed 14,000 organisms per c.c. after 4 months while the potency remained unaffected. The results indicate a very promising line of study and further experiments with better method of grinding are in progress.

XXIV. Action of Phenyl mercuric nitrate and Parachlormetacresol on Rabies Virus

(SAVOOR AND R. S. RAO)

As phenyl mercuric nitrate and parachlormetacresol are excellent bactericidal agents and act in very high dilutions, it was decided to see whether these chemicals would kill the rabies virus in similar dilutions. Phenyl mercuric nitrate was tested in 3 dilutions, 0.5, 1.0 and 5.0 mg. per 100 ml. and parachlormetacresol in two strengths of 10 and 20 mg. per 100 ml.

The method of preparation of vaccine was precisely the same as that used for the routine manufacture of phenolised rabies vaccine. To 8 per cent suspension of brain virus the above-mentioned chemicals were added and the suspension was incubated at 37°C. for 24 hours and then diluted to get a 5 per cent suspension. Phenolised vaccine made from the same brain suspension was used as a control in each instance. After storage at 0°C. for a period of one month, the vaccines were inoculated into rabbits and mice by the intra-testicular and intra-cerebral routes. Three separate experiments were carried out and it was found that these chemicals did not destroy the rabies virus. The vaccines prepared with phenyl mercuric nitrate, and parachlormetacresol provoked typical infection.

XXV. Typhoid Fever in the City of Bombay

(SOMAN)

During two years under report, free diagnostic aid to private practitioners for the diagnosis of enteric fever was continued. In all 811 and 1,866 blood samples were received for Widal test during the years 1942 and 1943 respectively. The marked rise in the total number of samples received during the year 1943, was due to the publicity given by the Institute to the proposed Typhus Inquiry.

The methods for the laboratory diagnosis of typhoid fever, essentially remained the same except a few modifications with regard to the treatment of contaminated clot-cultures, and the media used for culturing the samples of faeces. In absence of bed-side blood-cultures the combined method of clot-culture and Widal reaction was found to be the only method of choice. Its successful claims are amply borne out by the isolation of causative organism in 252 samples, during the year 1943, 146 of which when tested serologically, failed to demonstrate any diagnostic titre. Isolation of the organism was possible in 48 hours in 90 per cent of culturally positive cases, while in 10 per cent of such cases, prolonged incubation up to 5 days was found necessary. In spite of these distinct advantages, the method occasionally failed, because of gross contamination of the clot-culture in bile. This drawback was minimised, to a certain extent, by repeated sub-cultures and plating on selective media such as the bismuth-sulphite-agar or the D. E. C. medium.

Widal Test :—The diagnostic titres of the Widal test remained the same as before. In a few suspected carrier cases, where it was possible to collect blood along with samples of stool and urine, Vi agglutination test was done, and 1 in 10 was taken as the diagnostic titre. No proved case of paratyphoid B infection was met with either serologically or culturally.

The results as obtained by the combined method of clot-culture and Widal reaction for the years 1942 and 1943 are given below in a tabulated form :—

Year	Total samples	Hospital samples	Private samples	Widal positive	Clot-cultures positive	
					B. typhosus	B. Paratyphosus A
1942 ..	811	601	210	268	132	15
1943 ..	1,866	1,447	419	345	229	23

Cultures of faeces and urine :—Wilson and Blair's bismuth-sulphite-agar was the routine selective medium adopted so far, but during the year 1943, D. E. C. medium of Panja was tried along with the routine media. The new medium compared very favourably with the Wilson and Blair's medium. MacConkey medium and the litmus-lactose-agar medium were found to be utterly useless. In all 37 samples of faeces were examined, out of which 35 samples were received from convalescent typhoid cases; *B. typhosus* was isolated from 11 samples only. The new D. E. C. medium has now been adopted as a routine selective medium in the isolation of intestinal pathogens.

The results are summarized in the following table :—

Number of samples found positive	D. E. C. positive	B. S. A. positive	MacConkey positive	L. L. A. positive
11	10	8	0	0

XXVI. The Value of D. E. C. Medium in the Isolation of Intestinal Pathogens

(SOMAN)

A comparison was made of the value of three media namely, MacConkey agar, litmus-lactose-agar and D. E. C. medium devised by Panja and Ghosh, for the primary isolation of dysentery, enteric and cholera organisms from faeces. A total of 196 samples were examined, out of which 65 samples gave positive isolations for one or other of the organisms. D. E. C. medium was responsible for 92 per cent of positive results. The results of the other two media were extremely poor. D. E. C. medium is easy to prepare and is clear and translucent. The three pathogens grew well on it as colourless colonies in 24 hours. The growth of *Bacterium coli* and other intestinal saprophytic organisms was greatly suppressed. Contamination of plates with fungi and sporeformers was rare. So far no single medium was found suitable for primary isolation of all intestinal pathogens; the D. E. C. medium meets these requirements. Following table illustrates the nature of organisms isolated and the number and percentage of positive stool cultures with the various media.

Causative organism	Total positive	D. E. C. positive	L. L. A. positive	Mac. positive
B. dysentery group	18	16	5	3
V. cholerae	35	33	19	28
B. paratyphosus A	1	1	1	1
B. typhosus	11	10	0	0
Total	65	60	25	32
Percentage positive	—	92.3	38.4	49.2

L.L.A. = Litmus-lactose-agar; Mac = MacConkey agar.

XXVII. Studies on Leptospirosis

(M. N. LAHIRI)

Human cases of Leptospirosis.—In accordance with the memorandum issued to the hospitals in the City of Bombay all cases of jaundice with fever were reported to the Institute. During the period under review 101 cases of fever with acute jaundice were studied; this number does not include the large number of cases which were referred to the Institute, but were not acute. Out of the 101 cases 24 proved to be authentic cases of leptospirosis, in each of which the diagnosis was established by laboratory investigations. Four of these positive cases had a fatal termination. These positive cases, however, do not represent the true incidence of this disease in this city, as only a few of the many cases that actually occur are admitted to the local hospitals. Further, recent observations suggest that cases of the milder form usually pass unrecognised, while a few more are wrongly diagnosed as one of the other types of jaundice. The causal organism was isolated only from twelve of these cases and in the remaining the diagnosis was based on serological tests.

For the isolation of the organism, blood culture is a very valuable method during the early part of the disease; but this could not always be carried out, as either the disease was not suspected early enough or the cases came under observation at a time when the infective period of the blood had passed. In one instance only it was possible to culture the blood on the seventh day of illness, and it yielded a good growth of *Leptospira*. In the remaining cases isolation of the organism, therefore, had to be made from urine by means of animal inoculation. Contrary to the findings of many workers, the method of daily inoculation of urine into young guinea-pigs proved to be a very useful procedure in the present study. The method is a labourious one and no doubt involves the sacrifice of a large number of animals. On an average 8 to 10 samples of urine were used for each case between the 9th and 21st day of illness. In majority of the cases only one sample proved infective. It is, therefore, evident that the leptospirae are excreted out intermittently. Certain samples even showing leptospirae under dark-ground illumination failed to produce infection in the animals. Furthermore, according to my observation certain guinea-pigs were found to be refractory to infection. All the strains of *Leptospira* isolated belonged to the "classical" group, and so far this was found to be the only serological type responsible for the infection.

Of the serological tests the agglutination reaction was found to be a method of great practical value. In every case the diagnosis was pronounced long before the isolation of the organism. Experience has shown that strains isolated locally must be incorporated in carrying out the test. Further, the test should be repeated not only to note the fluctuation of titre but also the endtitre. From each case three samples were collected, the last sample usually was taken at the end of the third week of illness. A titre of 1 : 100 was found to be definitely suggestive of infection, as sera from many patients in whom the disease could be excluded failed to react even in a low dilution such as 1 : 20. In majority of the positive cases the final titre rose very high i.e. beyond 1 : 100,000.

Non-icteric form of leptospirosis.—The occurrence of the non-icteric type of cases has not as yet been recorded in India. Therefore, particular attention was directed to this question. So far only one positive case has been recognised by me in which there was no evidence of jaundice. The examination of the urine in this case showed total absence of bile. This finding suggests that such type of cases must have been missed before. With a view to throw further light

on the incidence of such form of the disease, samples of sera were examined for the presence of agglutinins which were sent to this Institute for routine serological reactions. Only those sera were picked out, which gave negative Widal reaction against the enteric group of organisms and the clot cultures were also found negative. In all, one hundred and fifty specimens of sera were tested out of which one showed a titre of 1 : 640, definitely diagnostic of infection. In carrying out the tests three local strains were used. The positive serum was not bile-tinted at all. These two instances indicate that cases of leptospirosis without icterus occasionally occur in the City of Bombay.

Maintenance of the virulence of the cultures of Leptospira.—It was observed that repeated subcultures resulted in a considerable loss of virulence. The virulence has, therefore, been maintained by passage through young guinea-pigs approximately every four weeks. In order to economise in animals the "Cryochem method"—drying from the frozen state—was studied. The substrata used were the defibrinated blood of guinea-pig and rabbit. After drying the cultures were regenerated at varying intervals and were examined under dark-ground illumination. It was found that even within forty-eight hours after drying the leptospirae had lost their motility and showed no evidence of any growth when inoculated into fresh medium. Similar results were obtained with the guinea-pig's liver emulsion showing leptospirae in considerable numbers. As no success was achieved the method was not considered suitable.

Transmission of Leptospira to mice.—In view of the recent observations of Larson in U. S. A. investigations were undertaken to determine the susceptibility of white mice towards *Leptospira icterohaemorrhagiae*. The mice bred at the Haffkine Institute were first employed. Two virulent cultures isolated from cases were selected. For each dose of the cultures, three white mice were used along with a young guinea-pig as a control. The mice were inoculated intraperitoneally with varying doses ranging from 0.2 to 0.6 c.c. of the cultures, whereas the control guinea-pig in each test received 1 c.c. Out of the thirty-six mice used in these experiments only one showed evidence of infection and the organism was recovered by culture of the heart's blood of the animal. The control guinea-pigs all died of the infection exhibiting typical post-mortem signs. Their liver emulsions also showed leptospirae in large numbers. Since different strains of mice might vary in their susceptibility towards leptospiral infection, Javanese strain of mice was used. Mice only three weeks old were employed. Out of thirty mice used in these experiments only two showed evidence of infection. These observations clearly indicate that both the strains of mice bred at the Institute are fairly resistant to the cultures of *Leptospira* isolated here. So far the experiments do not lend support to Larson's findings.

XXVIII. Studies on Typhus

(SAVOOR)

1. *Typhus in the City of Bombay.*—In 1940, Patel (T. B.) had reported 15 cases of Typhus in the City of Bombay. But interest in the disease again waned and the impression persisted that typhus occurred rarely, if at all, in the City. Early in 1943, the samples of sera sent to the Institute for Widal reaction were examined and 15 gave positive Weil Felix reaction. Similarly, during one month, samples of sera from exanthematous cases and those suffering from continued fever gave two positive Weil Felix reaction.

Through the co-operation of the Health Officer, Bombay Municipality, rats were trapped from two houses where cases of fever had given positive Weil Felix reaction. The brains of 23 rats so trapped were passaged in batches of four into guinea-pigs. One attempt succeeded. This strain produced the Neil-Mooser reaction, and showed plenty of *Rickettsia mooseri* in the serosal cells of the tunica. Unfortunately, the strain was lost as a result of a concomitant infection with *Toxoplasma caviae*. However, convalescent guinea-pigs, after infection with this strain showed complete protection against the Mysore strain (*vide infra*).

Three attempts were made to isolate strains from patients early in the course of disease. One attempt succeeded.

The procedure for isolating the virus consisted in inoculating intraperitoneally guinea-pigs and mice with 5 c. c. and 0.5 c. c. of blood respectively. The guinea-pigs used had been placed for six days on food autoclaved for two hours to render it vitamin-free. The strain so isolated produced in guinea-pigs, fever and tunica reaction (Neil-Mooser) in the male. The infected rabbits gave a Weil Felix reaction with *B. proteus* OX 19 and to a less extent with *B. proteus* OX 2. Both white rats and mice were susceptible to the virus. The virus, however, did not kill the animals. The virus remained alive for a period of over one month in the mouse. Three experiments were conducted which showed that the strain was easily transmitted by *X. cheopis*. There was a reciprocal cross-immunity with the Mysore typhus strain. These observations show that the strain isolated is probably flea-borne X 19 murine typhus.

On the basis of these observations, it was suggested to the Superintendents of local hospitals that typhus was perhaps not so rare in the city as it was believed to be. They were requested to co-operate in an investigation by sending samples of sera from cases of continued fevers. During the last six months of 1943, forty-four of the sera thus received have given positive Weil Felix reaction.

2. *A Strain of Typhus from Mysore*.—Drs. Heilig and Naidu from Mysore sent guinea-pigs, inoculated with material from two cases of typhus, for identification. From one of these, a strain was isolated and studied. In guinea-pigs, this strain gave the Neil-Mooser reaction, showed the presence of rickettsia in large number in the serosal cells of the tunica. Morphologically, these rickettsiae resembled *R. mooseri*. The virus survived in rats for twenty generations by brain and peritoneal passages, indicating a murine origin (Nicolle). But, the virus died in mouse passages within three generations, whether the route of inoculation was the brain or the peritoneum. The strain was easily transmissible by *X. cheopis*, and it cross-immunised with the strain isolated in Bombay.

It is of interest to note that Dr. Topping to whom the serum of this patient was sent by Drs. Heilig and Naidu found that the serum showed the presence of antibodies more nearly similar to those seen in Rocky Mountain spotted fever than in case of American endemic typhus. In view of this, it appears that the Mysore strain of typhus may not be identical with any of the known strains of typhus, but may be a form of typhus intermediary between the well-known murine strain and the Rocky Mountain spotted fever strain.

3. *A method of bed-side diagnosis of Typhus*.—Since the outbreak of this war, rapid bed-side methods of diagnosing typhus have been described. They all consist in carrying out an agglutination reaction on a slide with *B. proteus* killed or alive, stained or unstained. In our hands, a suspension in 0.5 per cent sodium citrate containing 0.2 per cent formalin made from *B. proteus* OX 19, killed with alcohol and stained with methylene blue, gave good results. For a positive reaction, the agglutination must occur in 15 seconds. The agglutinating properties

If the suspension lasted for three months if kept in the cold. For field diagnosis, this reaction seems to be particularly useful. Using the standard method of Weil-Felix reaction as a control a number of specimens were tested, no false reactions were obtained.

4. *Isolation of strains of XK Typhus*.—A localised outbreak of typhus had occurred in Assam and the Weil-Felix reaction had shown it to be due to XK virus. The Military Medical authorities requested the Haffkine Institute to send a Typhus Unit to Calcutta to isolate the strains responsible, with a view to make available for the production of a vaccine. The Unit successfully isolated three XK strains. The experimental animals used for isolation were the rabbit and the mouse, it is known that guinea-pig is not a suitable animal for the study of XK type of typhus. The strains so isolated have been sent to the American Typhus Commission at Cairo.

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