



REPORT
OF
THE HAFFKINE INSTITUTE

For the Years 1947-1948

BY
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CONTENTS PART I GENERAL

	Page
I. Staff	1
II. General Review	3
III. Department of Vaccines	5
<i>A. Plague Vaccine</i>	5
<i>B. Cholera Vaccine</i>	8
<i>C. T. A. B. Vaccine</i>	9
IV. Department of Antitoxins and Sera	9
V. Department of Entomology	12
<i>A. Examination of Bombay City Rats</i>	13
<i>B. Examination of Rats from the Bombay Port Trust Docks</i>	20
<i>C. Examination of Rats from Steamers and other Sources</i>	22
VI. Department of Clinical Pathology	22
<i>A. Bacteriological Section</i>	22
<i>B. Section of Public Health Chemistry and Bacteriology</i>	24
VII. Department of Pharmacology	25
VIII. Department of Chemotherapy	27
IX. Department of Biochemistry	29
X. Department of Nutrition and Experimental Pathology	30
<i>A. Nutrition Section</i>	30
<i>B. Pathology Section</i>	31
XI. Department of Virus Diseases including Rabies	35
XII. Blood Bank Department	40
XIII. Training of Health Personnel	42
XIV. Critics Club	44
XV. Income and Expenditure	45

PART II RESEARCH WORK

I. Sulphonamides and Antibiotics in the Treatment of Plague— Experimental Infection	46
II. Sulphonamides and Antibiotics in the Treatment of Plague— Clinical Trials	49

	Page
III. Sodium Phthalyl Sulphacetamide in the Treatment of Cholera Cases	54
IV. Titration of Anti-human Serum	55
V. Use of Tween-80 for Improvement of Solubility of Lyophilised Serum	56
VI. Use of Flocculation Test for Preliminary Assay of Anti-snake-venom Serum	56
VII. On the Pulicidal Effect of DDT	57
VIII. On the Pulicidal Effect of "Gammexane"	58
IX. On the Rodenticidal Effects of Dethdiet Red Squill and Rodine	58
X. Synthesis of Asymmetrical Diaryl Sulphones and Aryl-quinolyl Sulphones	59
XI. Synthesis of some Sulphanilamide and Sulphone Derivatives of Thiazole	59
XII. A Study of the Methods of Synthesis of Diguanydes	60
XIII. Synthesis of possible Lipophilic Chemotherapeuticals of the Sulphanilamide and Sulphone Series derived from Fatty Acids, including those of the Chaulmoogra Group	60
XIV. Synthesis of Phthalyl and ortho-Toluoyl Derivatives of the Sulphanilamides and Sulphones as possible Mycobacterial Antagonists	61
XV. Preparation of 5:5'-Dichlorosalicil and Heterocyclic Derivatives from Dichlorosalicil and Related alfa-Diketones	61
XVI. Thiazole Derivatives	62
XVII. Metabolism of <i>Penicillium Chrysogenum</i>	62
XVIII. Typhoid Fever in the City of Bombay	63
XIX. Cholera in Bombay City	64
XX. Typhus in Bombay City	66
XXI. Quantitative Determination of p-p' Dichloro-Diphenyl-Trichloro-ethane in Commercial DDT Dusts and Powders	69
XXII. The Effect of restricted Intake of Protein (Casein) in an otherwise adequate Diet on the Histological and Biochemical Changes in the Liver of Rats	69
XXIII. Experimental production of Hepatic Cirrhosis in Rats by High Fat-Low Protein and High Carbohydrate-Low Protein Diets and the Effect of Lipotropic Factors like Choline and Methionine on the Hepatic Lesions	70
XXIV. Treatment of Decompensated Portal Cirrhosis with High Caloric, High Protein Diets with Vitamin Supplements	72
XXV. On the Nutritive value of Vanaspati	72
List of Papers published from the Haffkine Institute during the years 1947-1948	75

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEARS
1947-48

PART I

GENERAL

I Staff

DIRECTOR

Major-Gen. Sir Sahib Singh Sokhey, M.A., M.D. (Edin.), D.T.M.&H. (Eng.), F.A.S., F.N.I.

Dr. P. M. Wagle, M.D. (Bom.), D.Bact. (Manch.), D.P.H. (Lond.), D.T.M. (Liverpool), B.M.S. (I), Officiated as Director from 15th March 1948 to 29th August 1948.

ASSISTANT DIRECTORS

Dr. P. M. Wagle, M.D. (Bom.), D.Bact. (Manch.), D.P.H. (Lond.), D.T.M. (Liverpool), B.M.S. (I), I/c Department of Vaccines.

Dr. D. C. Lahiri, M.B. (Cal.), D.T.M.&H. (Eng.), D.Bact. (Lond.), B.M.S. (I), I/c Department of Antitoxins and Sera.

Dr. A. K. Hazra, M.B.B.S. (Bom.), D.T.M. (Cal.), B.M.S. (I), Officiated as Assistant Director I/c Department of Antitoxins and Sera from 26th January 1947 to 16th November 1947.

Mr. B. V. Patel, B.Sc. (Bom.), B.Pharm. (Lond.), Ph.C. (Gr.Brit.), M.P.S., Officiating Assistant Director I/c Department of Pharmacology from 1st April 1947.

Dr. K. Ganapathi, B.A., D.Sc. (Madras), B.M.S. (I), I/c Department of Chemotherapy.

Dr. M. Sharif, D.Sc. (Punjab), Ph.D. (Cantab.), F.N.I., B.M.S. (I), I/c Department of Entomology up to 14th October 1948.

Dr. M. V. Radhakrishna Rao, M.B.B.S., Ph.D. (Andhra), F.N.I.,
B.M.S. (I), I/c Department of Nutrition and Experimental Pathology.

Dr. D. W. Soman, M.B.B.S., B.Hy. (Bom.), D.P.H., (R.C.P.S.),
D.T.M.&H. (Lond.), B.M.S. (I), I/c Department of Clinical Pathology.

Dr. R. Sanjiva Rao, M.B.B.S. (Madras), D.T.M. (Liverpool),
B.M.S. (I), I/c Department of Virus Diseases including Rabies up to
20th June 1948.

Lt. Col. J. R. Dogra, M.D., Ch.B. (Edin.), D.T.M. (Liverpool),
I/c Department of Virus Diseases including Rabies from 21st
June 1948.

II. General Review

A comprehensive review of the extensive development of the work of the Institute in recent years was given in the last report. Same trends remained in operation in the two years now under review. The production of biologicals steadily increased and brought the sales to thirty lakhs of rupees in 1948. The production of plague and cholera vaccines in 1947 rose to the huge total of 40 million millilitres, 23·5 million millilitres of plague vaccine and 16·5 million millilitres of cholera vaccine. This quantity was five times the average annual production in the previous quinquennium. The recent carefully planned reorganisation and re-equipment of the Department of Vaccines permitted the Institute to produce these huge quantities and meet all demands promptly. This large productive capacity proved of value also to some foreign countries, as the major part of the demand for cholera vaccine came from abroad and 10 million millilitres of the vaccine were shipped, mostly by air, to Egypt, Iraq and Siam. In 1948 also, the demand for these vaccines remained high and totalled 22 million millilitres, of which 16 million millilitres were of plague vaccine.

It is to be noted that the use of plague vaccine (formaline-killed) has been steadily increasing as a prophylactic measure since 1940. India uses now almost 16 million millilitres of it a year as against 4 million millilitres a year in 1940 and earlier. This, of course, is not due to any increase in the incidence of the disease in the country. On the contrary, the plague pandemic is in its declining phase and the total incidence of the disease as judged by the recorded deaths, the only data available, has been steadily declining; deaths from plague in British India during the five-year period 1935-1940 were 104,617 and in the next five years up to 1945, were 77,463. The increase in the use of plague vaccine (formaline-killed) is due to the increasing recognition of its high protective power by the Public Health Authorities and the public, coupled with the fact that the new vaccine issued since 1944 is almost atoxic and twice as potent. Its mouse lethal dose is 0·6 ml. as compared with 0·2 ml. of the previous vaccine and its mouse protective dose is 0·002 ml. as compared with 0·004 ml. of the previous vaccine. There has been a steady improvement in the potency of the vaccine since 1932 when its mouse protective dose was about 0·016 ml. It is, however, to be regretted that Indian conditions have not permitted the use of a multiple dose for mass inoculations. Inoculations are still carried out with a single dose. Even this is not done systematically and regularly. Inoculations are carried out when plague breaks out in an area, and there may be an interval of several years before the same individual living in an endemic area gets another single dose inoculation. Even with this unsatisfactory use of the vaccine, some figures collected in the field showed that the present vaccine used in a quantity of 2 ml. as a single dose inoculation reduced the attack rate to 1/3 and mortality rate to 1/9. This is high protection indeed, but investigations in experimentally-produced infection in mice showed that if the vaccine was given in two doses, protection was much greater. To get the same degree of protection in mice vaccine, if given in a single dose,

must be 3 times the total quantity of vaccine given in two doses. 0.01 ml. of the vaccine had to be used in a single dose to get the same protection as with two doses of 0.0015 ml. We feel sure, on the basis of our studies in experimental infection, that two dose inoculations followed by booster doses at regular intervals would render individuals almost immune to plague infection. But because we are aware of the difficulties involved in the practice of two dose inoculations in India today, we are now recommending that when single dose inoculation is practised 3 ml. of the vaccine should be given to an adult in place of 2 ml. dose recommended before. This increased quantity of vaccine will improve its immunising power. We have satisfied ourselves that this larger quantity of 3 ml. of our new detoxicated vaccines is well borne and gives little local or general reaction.

Just as in the case of vaccines, the use of antitoxins and sera has been steadily increasing. The Department of Antitoxins and Sera was organised in 1942 with a complement of 130 horses expected to yield twice the quantity of antitoxins then used in the whole of India. But with the availability, at a low cost, of antitoxins of high quality which give little or no serum reactions, the use of these biologicals has been steadily increasing all over the country, so much so that the new department with its maximum production can now only meet the needs of Bombay Province alone. This situation demands immediate ten-fold increase in production. Plans are being prepared to this end. The Department has continued its work on antiallergic serum and the clinical reports from the local fever hospital show that with antitoxins and sera treated with antiallergic serum the serum reactions are as low as 3 per cent. It is this reduction in serum sickness and serum shock with the use of the Institute antitoxins and sera that the demand for them has been increasing very rapidly.

The Department of Chemotherapy has successfully worked out with a modern pilot plant, economic methods for large-scale production of sulpha drugs and of the more important antimalarials. This work will prove of great value to the country. Based on this work the manufacture of sulphathiazole in sufficient quantities to meet the needs of the hospitals and public health authorities of the province has been started. Sulphathiazole is being issued at Rs. 25 a lb. This price is less than $\frac{1}{3}$ the price at which the public institutions could purchase their supplies from commerce. Work on these lines for other products is proceeding.

In July, 1947, Dr. M. V. Radhakrishna Rao, Assistant Director, in charge of the Department of Nutrition and Experimental Pathology was sent abroad to study the various measures adopted in different countries to improve the nutritional status of the population. During a period of one year he contacted the leading scientists in the U. K., the U. S. A., Canada and Australia, visited their laboratories and institutions, and studied the recent developments, both in laboratory technique and field investigations in nutrition. He has fully informed

himself of the field work being done in various countries he visited and studied the organisation of nutrition clinics, school feeding schemes and training of nutrition workers. He returned to India in August, 1948.

Since free diagnostic aid to general practitioners was instituted the work of the Department of Clinical Pathology has increased four-fold. Now over 25,000 samples are examined per year. The Department has also done valuable work on incidence of typhus fever in the City of Bombay. It has now been definitely established that murine typhus occurs all the year round in the city. Some cases of scrub typhus have been identified. These cases were spotted in the months of September to November.

The Department of Entomology continued its important researches on the ecology of Indian rat-fleas and proved a valuable training centre. Pharmacologists seem to be rare people, but every effort is being made to find and engage men of standing in this scientific discipline to man the department adequately to enable it to take up work necessary to implement the Drugs Act.

III. Department of Vaccines

During the years under review, large demands for plague and cholera vaccines were made on this department. The demands for the year 1947 were unprecedented and the largest for any year so far in the history of the Institute. They amounted to more than 23 million millilitres for plague vaccine and over 16 million millilitres for cholera vaccine. The demands for cholera vaccine, however, came largely from foreign countries. Egypt was supplied 3 million millilitres and Siam 4 million millilitres. In the year 1948 also, the demands were high, amounting to 16 million millilitres for plague vaccine and more than 6 million millilitres for cholera vaccine.

The demands came, as usual, from all over India, indicating the increasing popularity of these vaccines with the public. A systematic manner of prophylactic inoculations is still necessary as already stressed in the previous report.

A. PLAGUE VACCINE

During the years under review, the quantities of plague vaccine issued monthly are detailed below :—

Months	Quantities in millilitres	
	1947	1948
January	2,801,198	1,962,196
February	2,356,153	384,368
March	3,628,210	2,239,194
April	4,078,609	2,529,854
May	2,664,413	1,797,884
June	1,114,685	1,013,787
July	568,038	289,827
August	90,000	336,627
September	947,000	698,400
October	1,466,606	391,275
November	1,975,600	838,497
December	1,787,270	3,668,281
Total	23,477,782	16,150,190

The issue of the plague vaccine from the Institute since its beginning in quinquennial periods given below will show the very great increase for its demand during recent years.

Years				Millilitres
1898-1901	...	...	...	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
1942-1946	...	...	...	44,528,208
1947-1948	...	...	...	39,627,972

The plague vaccine is issued to India and other countries from this Institute. The demands from within and without India met during the years under review are shown below :—

					Quantities in millilitres	
					1947	1948
INDIA						
Bombay Province	..	..	..	..	2,789,304	2,005,809
Madras Province	..	..	..	..	1,842,560	806,665
Bengal, Bihar and Assam	..	..	..	..	3,630,700	..
West Bengal, Bihar and Assam		..	..	..	..	3,574,792
United Provinces		..	..	..	6,487,695	6,072,577
Punjab	..	..	..	..	1,641,455	..
East Punjab	..	..	..	..	..	144,460
Orissa	..	..	..	..	..	16,012
North West Frontier Province	..	..	..	..	5,800	..
Central Provinces and Berar	..	..	..	..	3,739,580	1,473,620
Delhi Province	..	..	..	..	511,340	51,290
Sind Province	..	..	..	..	16,000	..
Total					20,664,434	13,945,225

				Quantities in millilitres	
				1947	1948
INDIAN STATES					
Southern Maratha States	..	..	..	203,128	35,000
Hyderabad Deccan	..	..	..	1,280,700	875,500
Mysore	..	..	..	4,800	5,680
Patiala	..	..	..	149,400	78,280
Jaipur	..	..	..	6,075	900
Indore	..	..	..	9,020	18,450
Cochin	..	..	..	12,000	720
Gwalior	..	..	..	56,150	13,200
Benares	..	..	..	95,000	13,920
Travancore	..	..	..	20	...
Jodhpur	..	..	..	...	1,040
Bhopal	..	..	..	60,000	112,000
Rewa	..	..	..	46,400	49,200
Panna	..	..	..	16,400	5,000
Chhatarpur	..	..	..	40,000	2,000
Alwar	..	..	..	16,000	...
Rajnandgaon	..	..	..	...	5,000
Udaipur	..	..	..	60	75
Nagod	..	..	..	20,695	2,000
Kalsia	..	..	..	12,000	...
		Total	..	2,027,848	1,217,965
FOREIGN COUNTRIES					
Portuguese Government	..	..	..	15,500	1,000
Burma	..	..	..	770,000	480,000
Pakistan	..	..	..	...	506,000
		Total	..	785,500	987,000
		Grand Total	..	23,477,782	16,150,190

B. CHOLERA VACCINE

During the years under review the quantities of cholera vaccine despatched monthly are given below :—

Month	Quantities in millilitres					
				1947	1948	
January	...	...	...	...	14,965	444,741
February	...	...	...	...	1,006,946	783,155
March	...	...	...	...	1,676,829	442,601
April	...	...	...	...	1,462,274	135,363
May	...	...	...	...	863,676	292,063
June	...	...	...	...	443,731	1,194,547
July	...	...	...	...	599,000	1,413,859
August	...	...	...	...	1,000,000	893,398
September	...	...	...	...	3,732,680	356,256
October	...	...	...	...	3,746,952	203,493
November	...	...	...	...	1,757,900	83,195
December	...	...	...	...	579,998	56,372
Total				...	16,389,951	6,304,043

Following quantities of cholera vaccine, included in the above table were supplied to foreign countries during the years 1947 and 1948 :—

Foreign countries					1947	1948
Siam	...	...	...	...	4,000,000	
Egypt	...	...	...	...	3,000,000	
Iraq	...	...	...	...	1,300,000	700,000
Pakistan	...	...	...	...	1,475,000	
Cyprus	...	...	...	...		30,000
Total					9,775,000	730,000

C. T. A. B. VACCINE

The quantities of T. A. B. Vaccine despatched monthly during the years under report are given below :—

Month						Quantities in millilitres	
						1947	1948
January	...	...	...	...	...	12,723	27,105
February	...	...	...	...	...	1,700	16,538
March	...	...	...	...	...	8,388	46,732
April	...	...	...	...	...	7,600	17,835
May	...	...	...	...	...	10,726	39,206
June	...	...	...	...	...	21,437	88,642
July	...	...	...	...	...	43,954	53,413
August	...	...	...	...	...		43,905
September	...	...	...	...	...	32,000	163,080
October	...	...	...	...	...	239,740	36,969
November	...	...	...	...	...	77,075	24,468
December	...	...	...	...	...	43,007	33,757
Total ...						498,350	591,650

IV. Department of Antitoxins and Sera

The production of Antitoxins, Sera, and Toxoids has remained fairly constant since the peak of production was reached during 1945-46 soon after completion of construction of all stables, paddocks, and air-conditioned laboratories. The amount, which is being produced annually, is now all that can be produced from 130 horses. The stables and paddocks have accommodation for 130 horses, and so also the equipments and the laboratory space, which are adjusted for processing efficiently the sera obtained from 130 horses. Hence, annual production has remained constant since 1945.

But the demand for supply has been rising from year to year almost in geometric progression. For instance, the amount that seemed sufficient a few years ago to meet the entire needs of this and other provinces and states, and also part of the needs of the fighting forces, is now hardly enough to meet the needs of the Province of Bombay only. With the availability of all these essential products at a very cheap price, the demands which were so far latent, are becoming more and more apparent from year to year. However, limited by our adopted scale of production, which is intended primarily to meet the

needs of the hospitals of Bombay Province, we were compelled to withhold supplies from many of our extra-provincial buyers. Much that we regretted it, we saw no way out of this predicament until we can considerably enlarge this department.

During 1947, Dr. Lahiri was deputed abroad to study the newly developed method of alcohol fractionation of proteins, with a view to applying this method for purification of antitoxins and toxoids. Though much of the details of the method, being more or less trade secret, was withheld from him, it is still considered worth-while to work out the details at the Institute. The low temperature working laboratory, which is necessary for alcohol-fractionation is now under construction. If the new methods prove successful, they will be brought into use and the new method will give much purer and more stable antitoxins and toxoids.

The anti-allergic serum was continued to be added to all therapeutic sera issued from the Institute. Available reports seem to indicate that much of the serum reactions has been prevented by the anti-allergic serum.

Epizootic lymphangitis of the horses continued to cause anxiety throughout the year 1947. Very vigorous preventive measures, including destruction of all infected animals, succeeded in controlling the epizootic, but only at the cost of 61 fully immunized horses.

1. *Horses*.—107 horses were used during 1947, and 139 during 1948, for the production of various anti-toxins and sera. A number of preliminarily immunized horses varying between 25 to 30 were always kept in readiness. These were meant to replace those that were bled out or died. One horse was kept for obtaining normal horse serum, which was required both for use in man and for the preparation of media etc. in the Institute. The horses were grouped as follows :—

Antitoxins	Number of horses used	
	1947	1948
1. Tetanus Antitoxin	49	52
2. Diphtheria Antitoxin	18	37
3. Anti-snake-venom Serum	17	13
4. Welchii Antitoxin	4	20
5. Septique Antitoxin	2	5
6. Oedematiens Antitoxin	2	2
7. Anti-plague Serum	6	7
8. Anti-dysentery (Shiga) Serum	4	2
9. Special Anti-allergic Serum	4	Nil.
10. Normal Horse Serum	1	1
	107	139

2. *Preparation of toxins and toxoids.*—All the different toxins and toxoids, prepared during the period, were used mainly for the purpose of immunization and hyperimmunization of horses. Some portion of the toxins of tetanus and diphtheria were specially reserved for the preparation of respective toxoids for human use, and another small portion of diphtheria toxin was matured and utilised for the production of Schick Toxin.

The following table shows the amount of each type of toxins prepared during the period under review :—

Types of Toxins and Toxoids					Quantity in litres	
					1947	1948
1. Tetanus	...	...	...	...	180	293
2. Diphtheria	...	...	...	...	105	180
3. Welchii	...	...	...	...	25	76
4. Septique	...	...	...	...	28	26
5. Oedematiens	...	...	...	...	13	6
6. Shiga	...	...	...	...		9

3. *Preparation of antitoxins and sera.*—The total volume of fully processed antitoxins and sera, which was made available for human use during the period, amounted to 1,250,480 ml. The quantities prepared during each year were as follows :—

Types of Antitoxins or Sera					Quantities in millilitres	
					1947	1948
Concentrated and purified antitoxins	..			..	318,430	649,100
Natural serum	..	..	..	..	162,990	119,960
					481,420	769,060
1. Tetanus antitoxin	..	..	..	..	176,050	350,200
2. Diphtheria antitoxin	..	..	..	..	56,420	116,990
3. Gas-gangrene antitoxin	..	..	..	..	43,830	104,710
4. Anti-snake-venom serum	..	..	..	..	42,130	77,200
Total concentrated sera	..			..	318,430	649,100
5. Anti-dysentery serum (natural)	..	..	..	..	11,280	22,670
6. Anti-plague serum	..	..	..	..	122,290	71,500
7. Anti-allergic serum	..	..	..	..	13,270	—
8. Normal horse serum	..	..	..	..	16,150	25,790
Total natural sera	..			..	162,990	119,960

4. *Lyophilization of Sera*.—During these two years a total volume of 153,470 millilitres of polyvalent anti-snake-venom serum was processed for preservation by drying from the frozen state. Out of these 34,140 ml. were carried over from 1946, 42,130 ml. were produced in 1947 and 77,200 ml. produced in 1948. After lyophilization, 55,140 ml. of serum was issued for use in man during 1947, and 54,500 ml. during 1948.

5. *Supply*.—The following quantities of antitoxins, sera and toxoids were issued during the years 1947-1948 :—

		Quantities supplied in millilitres	
		1947	1948
1.	TETANUS ANTITOXIN		
(a)	Prophylactic—3,000 I. U. in 2 c.c.	1,21,364	1,84,032
(b)	Curative—20,000 I. U. in 10 c.c.	1,24,200	1,53,750
(c)	Curative—10,000 I. U. in 5 c.c.	8,165	5,775
(d)	Highly concentrated—20,000 I. U. in 4 c.c.	1,588	25,024
2.	DIPHTHERIA ANTITOXIN		
(a)	Prophylactic—1,000 I. U. in 2 c.c.	2,684	1,738
(b)	Curative—10,000 I. U. in 10 c.c.	68,900	68,760
(c)	Curative—5,000 I. U. in 5 c.c.	2,905	1,760
(d)	Highly concentrated—10,000 I. U. in 5 c.c.	345	2,230
3.	GAS GANGRENE ANTITOXIN		
(a)	3,000 I. U. Welchii antitoxin in 10 c.c.	47,670	51,820
(b)	9,000 I. U. Welchii, 4,500 I. U. Septique and 9,000 I. U. Oedematiens antitoxins in 51 c.c.	30	2,400
4.	ANTI-PLAGUE SERUM in 20 c.c.	1,01,680	39,380
5.	LYOPHILIZED POLYVALENT ANTI-SNAKE-VENOM SERUM in 10 c.c.	55,140	54,500
6.	ANTI-DYSENTERY SERUM		
(a)	Natural, 4,000 I. U. in 20 c.c.	8,680	3,880
(b)	Concentrated, 4,000 I. U. in 5 c.c.	925	690
7.	TETANUS TOXOID		
(a)	in 10 c.c.	11,070	9,630
(b)	in 5 c.c.	3,005	760
(c)	in 1 c.c.	404	673
8.	DIPHTHERIA TOXOID ALUM PRECIPITATED		
(a)	in 5 c.c.	425	65
(b)	in 1 c.c.	176	319
9.	LYOPHILIZED SCHICK TOXIN Sufficient for 25 tests.	3	
10.	NORMAL HORSE SERUM in 10 c.c.	—	3,690

6. *Training*.—Mr. H. M. Pandya followed the working of the animal room, particularly with regard to the breeding and upkeep of the laboratory animals. Mr. D. C. Katugampola worked on the production of diphtheria toxoid, and Dr. P. S. Unalivia on the production and standardization of therapeutic antitoxins. Mr. S. V. Punnambalam took training in the preparation of diphtheria toxoid for human immunization. In addition, they all followed the routine working of the department.

V. Department of Entomology

Besides the usual routine work, this department was engaged in researches on fleas and rodents and their control. Three papers on the ecology of Indian rat-fleas, which were accepted for publication in the United Kingdom were revised and their proofs corrected. Further

experiments on the lasting effects of different formulations of DDT and 666 were conducted. The rodenticidal value of Dethdiet Red Squill and Rodine were determined. The results of these researches are summarized in Part II of this report.

In addition, the department was engaged in compilation, statistical calculation and tabulation of the flea and rodent data collected during the period of field-investigations to ascertain the causes of plague re-crudescence in the Southern Registration Districts of the Bombay Province. The plague infected places in the Bombay Province for sixteen years, *i.e.* 1930-45, were tabulated taluka-wise.

During the years under review inquiries about fleas, ticks and rodents and their control were attended to. Samples of rat-traps were received for examination and expert advice from different local bodies and manufacturing firms. Suggestions to improve their construction were given to them.

A fortnightly course of training in plague and anti-plague measures was given to 32 Sanitary Inspectors and one Medical Officer deputed by different Local Bodies of the Bombay Province. Short courses of instruction were given to 1947 and 1948 classes of Sanitary Inspectors and health visitors. Mr. S. K. Sobti, M.Sc., Entomologist to the Public Health Department of Central Provinces and Berar and Dr. G. R. Maw-lankar, Medical Officer in charge of Rat Campaign, Poona City Municipality, were given training in plague and anti-plague measures. Mr. P. N. Ranga Rao, B. Sc., deputed by the Public Health Department of the Bombay Province, was trained for a fortnight in the identification of fleas, flea counts, plague survey technique, and the use of DDT as antiplague measure so as to enable him to conduct field trials with different formulations of DDT. Assistance was given to the Public Health Department of this Province in the purchase of apparatus required for these field trials.

A. EXAMINATION OF BOMBAY CITY RATS

A permanent organization 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 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 any part of the city with a view to detect and control plague in it.

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, 44 plague infected rats were detected.

TABLE I.—*Showing rats received at the Institute from the Bombay Municipality during 1947-1948*

Month	1947			1948		
	Alive	Dead	Total	Alive	Dead	Total
January	... 7,313	40,553	47,866	13,682	55,465	69,147
February	... 10,516	63,251	73,767	11,180	52,942	64,122
March	... 11,629	69,433	81,062	15,433	65,868	81,301
April	... 9,155	63,891	73,046	20,648	85,155	1,05,803
May	... 8,936	60,915	69,851	16,739	76,257	92,996
June	... 7,609	60,779	68,388	12,587	85,204	97,791
July	... 10,487	67,860	78,347	9,981	72,192	82,173
August	... 11,745	65,502	77,247	15,512	76,634	92,146
September	... 9,191	60,163	69,354	19,413	70,613	90,026
October	... 10,044	54,587	64,631	24,952	70,176	95,128
November	... 9,020	47,643	56,663	27,898	61,228	89,126
December	... 11,435	60,697	72,132	33,534	64,808	98,342
Total	... 1,17,080	7,15,274	8,32,354	2,21,559	8,36,542	10,58,101

TABLE II.—*Showing dead rats examined for plague during 1947-1948*

Month	Dead rats examined		Found plague-infected in 1947	Found plague-infected in 1948			
	in 1947	in 1948		<i>Rattus norvegicus</i>	<i>Rattus rufescens</i>	<i>Rattus rufescens</i>	<i>Gunomys Kok</i>
January	... 27,122	25,610	<i>Nil</i>	<i>Nil</i>		<i>Nil</i>	<i>Nil</i>
February	... 41,659	18,754	...	...		...	...
March	... 44,586	31,068	...	...		1	1
April	... 27,933	43,752	...	...		1	<i>Nil</i>
May	... 21,904	38,962	...	...		<i>Nil</i>	1
June	... 22,294	31,212	...	...		...	<i>Nil</i>
July	... 18,226	32,644	...	...		...	..
August	... 17,771	25,425	...	...		...	..
September	... 16,069	27,246	...	...		..	.
October	... 20,089	28,475	...	...		...	25
November	... 20,458	27,108	...	...		...	13
December	... 29,741	28,765	...	...		...	2
Total	... 3,07,852	3,59,021*	<i>Nil</i>	<i>Nil</i>		2	42

*In addition to dead rats 1,161 live rats from the plague infected localities were examined during the year 1948. The total number of live and dead rats examined in 948 is 3 60 182.

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

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> .	1,218	20·2	1,427	17·1	1,590	17·6	1,346	18·1	1,488	20·3	1,175	22·0
<i>Rattus norvegicus</i> ..	1,441	23·9	1,875	22·4	2,213	24·5	1,809	24·3	1,639	22·3	1,449	24·7
<i>Gunomys kok</i> ..	2,956	49·0	4,443	53·1	4,680	51·7	3,854	51·7	3,759	51·2	2,901	49·4
<i>Bandicoota malabarica</i>	106	1·8	132	1·6	157	1·7	51	0·7	20	0·3	14	0·2
<i>Mus dubius</i> ..	115	1·9	153	1·8	144	1·6	176	2·4	143	2·0	116	2·0
<i>Suncus caeruleus</i>	196	3·2	336	4·0	259	2·9	212	2·8	288	3·9	220	3·7
*Total ..	6,032	100·0	8,366	100·0	9,043	100·0	7,448	100·0	7,337	100·0	5,875	100·0

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

*These totals do not agree with the totals of live rats in Table I, because rats received on Sundays

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

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> .	1,782	17·0	1,107	17·2	2,828	23·7	4,085	23·9	2,555	18·1	2,285	21·8
<i>Rattus norvegicus</i> ..	2,088	19·9	1,340	19·2	2,329	19·5	3,251	19·1	2,841	20·1	2,551	24·3
<i>Gunomys kok</i> ..	5,872	56·0	4,036	57·9	5,879	49·2	8,413	49·4	7,772	55·1	4,986	47·6
<i>Bandicoota malabarica</i> .	47	0·5	76	1·1	125	1·0	182	1·1	201	1·4	16	0·2
<i>Mus dubius</i> ..	270	2·6	115	1·7	224	1·9	304	1·8	277	2·0	267	2·5
<i>Suncus caeruleus</i> ..	418	4·0	204	2·9	565	4·7	808	4·7	463	3·3	380	3·6
§Total .	10,477	100·0	6,968	100·0	11,950	100·0	17,013	100·0	14,109	100·0	10,485	100·0

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

*These totals do not agree with the totals of live rats in Table I because rats received on Sundays

rats trapped alive by Municipal Corporation, Bombay, during 1947*

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
1,553	17.7	1,416	16.3	1,473	22.0	1,458	19.8	1,444	22.9	1,473	17.5	17,061	18.9
1,932	22.1	1,888	21.8	1,633	24.4	1,729	23.4	1,414	22.4	1,748	20.7	20,770	23.0
4,823	55.0	4,852	56.0	3,220	48.0	3,792	51.4	3,033	48.9	4,672	55.5	47,035	52.0
10	0.1	7	0.1	9	0.1	2	0.0	3	0.0	26	0.3	537	0.6
161	1.8	173	2.0	150	2.2	149	2.0	152	2.4	175	2.1	1,807	2.0
286	3.3	332	3.8	224	3.3	250	3.4	210	3.4	331	3.9	3,144	3.5
8,765	100.0	8,668	100.0	6,709	100.0	7,380	100.0	6,306	100.0	8,425	100.0	90,354	100.0

tute dead often mutilated and even putrid.

and holidays were not classified.

rats trapped alive by the Municipal Corporation, Bombay, during 1948*

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
1,900	22.4	2,415	21.3	3,346	22.6	5,397	28.1	6,438	29.9	7,143	25.2	41,371	23.7
2,014	23.8	2,779	24.5	3,020	20.4	2,865	14.9	2,616	12.2	4,395	15.5	32,089	18.3
3,924	46.3	5,240	46.1	6,575	44.3	8,790	45.7	9,645	44.9	11,724	41.3	82,856	47.4
20	0.2	27	0.2	156	1.0	113	0.6	230	1.1	249	0.9	1,442	0.8
213	2.5	289	2.5	582	3.9	778	4.0	798	3.7	1,768	6.2	5,885	3.4
402	4.8	611	5.4	1,150	7.8	1,283	6.7	1,765	8.2	3,094	10.9	11,143	6.4
8,473	100.0	11,361	100.0	14,829	100.0	19,226	100.0	21,492	100.0	28,373	100.0	174,786	100.0

tute dead, often mutilated and even putrid.

and holidays were not classified.

TABLE V.—*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>	5,292	15.5	7,803	15.7	8,235	15.7	4,258	8.1	2,921	5.7	3,145	0.5
<i>Rattus norvegicus</i>	.. 7,005	20.7	11,758	23.0	12,134	23.1	7,023	13.3	5,258	10.3	5,063	11.7
<i>Gnomys kok</i>	.. 13,332	39.0	20,412	41.1	22,317	42.6	14,046	26.5	11,361	22.3	11,692	24.3
<i>Bandicoota mamlabarica</i>	157	0.5	157	0.3	165	0.3	258	0.5	194	0.4	235	0.6
<i>Mus dubius</i>	.. 1,276	3.7	1,529	3.1	1,735	3.3	2,348	4.4	2,170	4.2	1,595	3.3
<i>Suncus caeruleus</i>	.. 1,504	4.4	1,835	3.7	2,052	3.9	2,205	4.3	2,002	5.1	2,654	5.5
<i>Putrid</i>	.. 5,542	16.2	6,219	12.5	5,812	11.1	22,091	42.0	20,555	52.0	23,215	48.2
*Total	.. 34,168	100.0	49,713	100.0	52,450	100.0	52,919	100.0	51,061	100.0	48,190	100.0

*These totals do not agree with the totals of dead rats in Table I, because rats received on Sundays

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>	5,812	14.2	4,144	12.8	5,716	11.5	8,851	13.1	7,865	12.3	6,728	9.5
<i>Rattus norvegicus</i>	.. 6,208	15.2	4,447	13.7	7,903	15.9	12,048	17.9	10,123	15.8	7,755	11.0
<i>Gnomys kok</i>	.. 11,136	27.2	8,247	25.5	15,372	30.9	20,055	29.8	18,329	28.6	14,171	20.0
<i>Bandicoota malabarica</i>	376	0.9	239	0.7	299	0.6	436	0.7	347	0.5	371	0.5
<i>Mus dubius</i>	.. 2,078	5.0	1,677	5.2	1,695	3.4	2,321	3.4	2,298	3.6	2,227	3.2
<i>Suncus caeruleus</i>	.. 2,555	6.2	2,134	6.6	2,310	4.7	3,556	5.3	3,604	5.6	3,405	4.8
<i>Putrid</i>	.. 12,813	31.3	11,515	35.5	16,400	33.0	20,106	29.8	21,532	33.6	36,000	51.0
*Total	.. 40,978	100.0	32,403	100.0	49,695	100.0	67,373	100.0	64,098	100.0	70,617	100.0

*These totals do not agree with the totals of dead rats in Table I, because rats received on Sundays

of rats found dead in the City of Bombay during 1947

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
2,975	5.2	3,051	6.4	2,937	6.6	3,156	7.8	3,705	11.3	5,970	13.4	53,448	9.0
4,422	7.7	3,978	8.3	3,898	8.8	4,658	11.5	4,691	14.2	7,235	16.2	77,783	14.0
8,943	15.7	8,684	18.1	7,407	16.7	9,923	24.5	10,405	31.6	13,071	29.3	151,593	27.3
246	0.4	245	0.5	144	0.3	391	1.0	279	0.8	326	0.7	2,797	0.5
1,640	2.9	1,813	3.8	1,683	3.8	1,961	4.9	1,378	4.2	2,779	6.3	21,907	3.9
2,531	4.4	2,656	5.5	2,356	5.3	2,767	6.9	2,217	6.7	3,288	7.4	28,666	5.2
36,405	63.7	27,512	57.4	25,901	58.5	17,674	43.7	10,264	31.2	11,896	26.7	219,686	39.5
57,162	100.0	47,939	100.0	44,326	100.0	40,439	100.0	32,939	100.0	44,565	100.0	555,880	100.0

and holidays were not classified.

of rats found dead in the City of Bombay during 1948

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
6,941	11.2	5,833	10.5	6,060	11.8	6,574	12.8	5,915	13.1	6,194	11.6	76,633	11.9
8,297	13.4	6,428	11.6	6,764	13.2	6,974	13.6	6,640	14.7	6,499	12.1	90,086	14.0
14,430	23.4	10,795	19.5	11,713	22.8	12,132	23.6	11,395	25.1	12,719	23.8	160,494	24.9
478	0.8	320	0.6	174	0.3	234	0.4	175	0.4	50	0.1	3,468	0.5
2,498	4.1	2,040	3.7	2,535	4.9	2,429	4.7	2,152	4.8	2,796	5.2	26,746	4.2
3,779	6.1	3,464	6.3	3,845	7.5	3,634	7.1	2,934	6.5	3,878	7.3	39,098	6.1
25,330	41.0	26,488	47.8	20,294	39.5	19,467	37.8	16,018	35.4	21,376	39.9	247,330	38.4
61,753	100.0	55,377	100.0	51,385	100.0	51,444	100.0	45,229	100.0	53,512	100.0	643,864	100.0

and holidays were not classified.

B. EXAMINATION OF RATS FROM

These rats were received along with those of the other wards of monthly totals given in previous tables. A separate record of these also maintained at the instance of the Public Health Commissioner. Tables VII-VIII 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> .	202	49.6	218	33.4	169	31.4	218	41.8	256	44.6	148	28.92
<i>Rattus norvegicus</i> ..	37	9.1	107	16.4	125	23.3	76	14.6	75	13.1	88	17.2
<i>Gunomys kok</i> ..	116	28.5	248	38.1	182	33.9	192	36.9	173	30.1	215	42.0
<i>Bandicoota malabarica</i> .	2	0.5	2	0.3	—	—	1	0.2	2	0.4	1	0.2
<i>Mus dubius</i> ..	18	4.4	10	1.5	16	3.0	12	2.3	23	4.0	16	3.1
<i>Suncus caeruleus</i> ..	32	7.9	67	10.3	45	8.4	22	4.2	45	7.8	44	8.6
*Total	407	100.0	652	100.0	537	100.0	521	100.0	574	100.0	512	100.0

*Rats received on Sundays and holidays were not classified.

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> .	289	46.5	215	39.9	134	25.9	226	35.0	207	33.1	246	36.9
<i>Rattus norvegicus</i> ..	81	13.1	126	23.4	114	25.1	103	15.9	141	22.6	156	23.5
<i>Gunomys kok</i> ..	201	32.4	152	28.2	146	32.2	233	36.0	188	30.1	191	28.7
<i>Bandicoota malabarica</i> .	—	—	2	0.4	2	0.4	7	1.1	4	0.6	1	0.2
<i>Mus dubius</i> ..	20	3.2	19	3.5	9	2.0	17	2.6	43	6.9	21	3.2
<i>Suncus caeruleus</i> ..	30	4.8	25	4.6	49	10.8	61	9.4	42	6.7	50	7.5
*Total	621	100.0	539	100.0	454	100.0	647	100.0	625	100.0	665	100.0

Rats received on Sundays and holidays were not classified.

THE BOMBAY PORT TRUST DOCK

the Bombay Municipality, and have been already accounted for in the rats, giving the number and percentage of the different species, is

of rats caught alive from the Bombay Port Trust area in 1947

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
158	27.2	159	34.2	223	49.0	186	36.6	200	43.7	174	42.0	2,311	38.0
113	19.4	72	15.5	72	15.8	98	19.3	72	15.7	53	12.8	988	16.2
260	44.7	158	34.0	98	21.6	184	36.2	154	33.6	132	31.9	2,112	34.7
—	—	—	—	1	0.2	1	0.2	—	—	2	0.5	12	0.2
17	2.9	23	4.9	15	3.3	10	2.0	7	1.5	6	1.4	173	2.9
34	5.8	53	11.4	46	10.1	29	5.7	25	5.5	47	11.4	439	8.0
582	100.0	465	100.0	455	100.0	508	100.0	458	100.0	414	100.0	6,085	100.0

of rats caught alive from the Bombay Port Trust area in 1948

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
245	37.0	190	37.5	224	37.5	205	36.9	139	30.0	243	36.2	2,563	36.6
97	14.7	86	17.0	111	18.6	115	20.7	64	13.8	126	18.8	1,320	18.8
253	38.2	172	33.9	192	32.2	163	29.3	151	32.5	183	27.2	2,225	31.8
6	0.9	2	0.4	—	—	3	0.6	—	—	4	0.6	31	0.4
15	2.3	19	3.7	19	3.2	23	4.1	25	5.4	42	6.2	272	3.9
46	6.9	38	7.5	51	8.5	47	8.4	85	18.3	74	11.0	598	8.5
662	100.2	507	100.0	597	100.0	556	100.0	464	100.0	672	100.0	7,009	100.0

C. EXAMINATION OF RATS FROM STEAMERS AND OTHER SOURCES

During the years 1947 and 1948, 975 and 684 dead rats and 58 and 44 smears from dead rats respectively were received from different towns of the Bombay Province. As a result of their examination 12 and 10 rats were reported upon as being plague infected in 1947 and 1948 respectively from towns mentioned in Table IX.

TABLE IX.—*Showing the different localities in the Bombay Province from which plague-infected rats were received during 1947-48*

Name of Town.	Number of plague infected rats.	
	1947	1948
Ahmednagar	...	3
Ambernath	...	2
Bail Hongal	...	1
Dasgaon (Kolaba)	1	...
Kankavali (Ratnagiri)	1	...
Khed	1	...
Mahad	1	2
Mangaon (Kolaba)	2	...
Nasik	1	...
Panvel	2	...
Sangamner	1	...
Shahpur (Thana)	2	...
Uran (Kolaba)	...	2
	12	10

During the years under review 916 killed rats from steamers were received from the Health Officer of the Port of Bombay and of these 612 belonged to the species *Rattus rattus rufescens*, 131 *Rattus norvegicus*, 168 *Mus dubius* and 5 *Suncus caeruleus*. None of them was found plague infected.

VI. Department of Clinical Pathology

A. BACTERIOLOGICAL SECTION

This section examined clinical material from various Government hospitals and dispensaries in the city and the mofussil in the province. In addition, private medical practitioners contributed a good number

of samples for diagnostic work. Free diagnostic aid is given to all practitioners in infectious diseases such as diphtheria, dysentery, cerebro-spinal fevers, enteric fever, typhus fever, cholera, malaria etc. In December, 1947, Government of Bombay sanctioned the typhoid scheme under which additional staff was appointed at the Haffkinè Institute to permit the Institute to send out a medical man to take samples of blood and other materials from patients suspected of enteric fever. The scheme also provided a car to facilitate the collection of blood. The scheme actually started working from middle of June, 1948 and in six months attended 468 calls all over the city and suburbs.

The following table gives an idea of some of the important examinations carried out during the years under report.

Nature of examination	1947		1948	
	No. of samples examined	No. of positive samples	No. of samples examined	No. of positive samples
Wassermann test	7,108	1,065	7,535	1,483
Kahn test	1,565	145	1,605	195
Widal test	1,587	359	2,191	640
Agglutination test for typhus fever	1,649	124	2,228	100
Agglutination test for Brucellosis	1,519	2	2,191	...
Sputum and urine for T.B. by smear and animal inoculation	225	48	248	82
C. S. F. smear and culture	80	4	87	9
Throat swab for diphtheria	59	15	98	16
Stool examination for V. cholera	1,657	Inaba 305 Ogawa 9	1,610	56
Stool for examination of intestinal pathogens other than V. cholera	883	404	700	485
Blood films for malaria	814	69	1,002	31
Friedman test for pregnancy including 24 Hour Rat Test	142	48	188	69
Rideal Walker Test	35	...	43	...
Autogenous Vaccines	35	...	33	...
Blood counts	401	...	517	...

The city was not free from cholera in the year 1947. First positive isolation of V. cholera was reported from a sample from Arthur Road Hospital in May. From July to December, 1947, nearly 1,600 samples

were examined and 314 positive isolations obtained. All the strains were identified as to their sub-types, of which 305 were found to be of Inaba and 9 of Ogawa. 232 samples of blood were examined for the diagnosis of Leptospiral infection, out of which 21 were positive serologically.

This section was also engaged in the maintenance of various species of poisonous snakes for the purpose of extraction of venom. The quantities of different venoms collected during these two years are given below :—

Year				Cobra venom in gms.	R. viper venom in gms.	Krait venom in gms.	Echis venom in gms.
1947	...	...	...	24·140	20·800	4·170	12·580
1948	...	...	...	68·00	19·450	4·203	6·299

The diagnostic reagents section carried on the work of preparing reagents for diagnostic purposes. The staff which was sanctioned on a temporary basis 4 years ago, was made permanent during the year 1948. The reagents are supplied to the laboratories all over India and the demands are rapidly increasing every year. The following reagents were supplied during the years under report:—

Reagents.				1947	1948
1.	Blood grouping sera	..	..	2,234 ml.	2,208 ml.
2.	High titre sera	..	..	711 ml.	518 ml.
3.	Killed suspension for Widal and Weil-Felix	..	..	76,000 ml.	96,000 ml.
4.	Kahn antigen	..	..	210 ml.	415 ml.
5.	Amboceptor	..	..	37 ml.	63 ml.
6.	J. S. B. Stain	..	..	16 lbs.	3 lbs.

B. SECTION OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY

This section mainly examined samples of waters for their fitness for drinking, sanitary and industrial purposes, samples of milk mostly sent by the Civil Supplies (Secretariat) for bacteriological and chemical grading and detection of adulteration, and other miscellaneous samples.

The following table gives a summary of the various examinations carried out during the two years under review :—

Samples examined	Nature of examination	Number of samples examined	
		1947	1948
Water ..	.. Fitness for drinking purposes ..	21	52
Water (aerated) ..	.. Do. ..	..	6
Water ..	.. Fitness for sanitary and industrial purposes ..	9	12
Water ..	.. Fitness for swimming purposes ..	2	2
Milk ..	.. Bacteriological and chemical grading and detection of adulteration. ..	51	17
Milk (condensed) ..	.. Fitness for human consumption ..	1	..
Sugar Syrup ..	.. Do. ..	1	..
Sewage ..	.. Sterility ..	..	3

VII. Department of Pharmacology

The Department continued to examine drugs for the military till the end of September, 1947. The enforcement of Drugs Act and Rules thereunder from 1st April, 1947, necessitated the establishment of Drugs Testing Section in the Department. The fitting up of the laboratories of the Department on the 2nd floor for the purpose of testing of drugs was taken in hand and the Department was shifted to the new floor in June, 1948.

The Department has undertaken the work of analysis of Schedule C & C(1) drugs on behalf of Bihar, Orissa, Assam, Central Provinces and Berar, East Punjab, Delhi and Ajmer-Merwara Provinces and for drugs other than Schedule C & C(1), on behalf of Central Provinces, Berar and East Punjab. The Department has also undertaken the analysis of drugs and chemicals imported into the country at Bombay under Drugs Rules 40.

1. *Examination of drugs, chemicals and apparatus on behalf of the military authorities.*—On account of cessation of hostilities the number of samples received from the Officer Commanding, Medical Stores, Inspection Depot, were considerably less than in the previous years. The samples that were received were mainly from the stocks to be disposed of by the army and, therefore, were sent for analysis. The samples were assayed both chemically and biologically for their compliance with the Pharmacopoeial Standards,

2. *Preparation of Sulphanilamide-Vitaminised Oil paste.*—The Department continued to manufacture this paste to supply the needs of the various Civil Hospitals of the Bombay Province. Medical Officers in the Province indented for this paste and all their demands during the two years were fully met. The total supplies made were 162 lbs. in 1947 and 272 lbs. in 1948.

3. *Standardization of drugs sent by manufacturers of Schedule C & C(1) products.*—The manufacturers sent their products for standardization to this Department. They consisted chiefly of Tincture Digitalis, Tincture Strophanthus, Adrenalin Solutions, Pituitary Extracts, and Liquid Extract of Ergot. Samples of organic arsenicals and antimonials were received for determination of their toxicity. Several chemicals were also received for their compliance with Pharmacopoeial Standards.

4. *Pyrogen Tests.*—The parenteral fluids prepared in the Institute were regularly tested for absence of pyrogens. Due to the enforcement of the Drugs Act number of commercial firms also sent samples of distilled water, glucose solution, normal saline etc., for pyrogen test.

5. *Examination of drugs and chemicals under the Drugs Act, 1940, and Drugs Rules, 1945.*—The work of testing of the samples from the Drugs Controller for the Province of Bombay was carried out. The number of samples received was not large. No Inspectors required for taking the samples had yet been employed. A number of samples of various preparations of Quinine such as powders, tablets, and ampoules were received through the Drugs Controller for the Province of Bombay. These had actually been forwarded by the Government of India for investigation and all of them were found to be completely devoid of quinine.

Samples of various quinine salts, sulpha drugs, chemicals etc., were received from the Assistant Collector of Customs, Bombay, and Cochin. During the period September to December, 1948, a total of 256 samples were examined. On the whole the products complied with the Pharmacopoeial Standards.

6. *Miscellaneous Work.*—The examination of drugs for their alleged curative action in snake bites was carried out and none of the drugs tested were found to be effective.

7. *Research Work.*—Pressure of routine work and the shifting of the laboratories to its new premises left little time to pursue research work. However investigations on the action of ions on the central nervous system were carried out in the Department. A method for the estimation of p-p'-dichloro-diphenyl-trichloro-ethane (D. D. T.) in dusts and powders has been developed. The problem of developing a laboratory method for the standardising preparations of liver extract has been taken on hand. The results of these investigations are given in Part II of this Report.

VIII. Department of Chemotherapy

The department continued the investigations outlined in the previous report both on the theoretical and applied side. Studies in the chemotherapy of tuberculosis and malaria were undertaken. In addition, the regular manufacture and supply of sulphathiazole has been started. The process of manufacture of the antimalarial paludrine has been studied and standardised and the Department is in a position to undertake, if required by the Government, the manufacture of about 30,000 lbs. of this drug per year.

Following the discovery and patenting of the new process of manufacture of sulphathiazole by the Department, the method has been standardised on a pilot plant scale. The Government has sanctioned staff to manufacture the drug and supply it to all the Government hospitals, dispensaries and public health institutions. So far 250,000 tablets and 1992 ampoules of the drug have been supplied. Since the drug is being sold at less than a third of the regular market price, the demand for it is increasing. When facilities permit, it is proposed to take-up the manufacture of sulphamerazine also. A process for the preparation of sulphathiazole which is anticipated to be an improvement over that now being employed has been discovered; the patent applied for this has also been accepted. Attempts are being made to work out the exact conditions for the adoption of this process on a commercial scale since this would result in a further reduction of the cost of production.

Malaria, being the most important problem facing our country, great deal of attention has been given to the manufacture of the antimalarials at low costs to enable Government to take up extensive antimalarial measures. The method of manufacture of the new drug, N¹-p-Chlorophenyl-N⁵-isopropyl diguanide hydrochloride (paludrine), which appears to be well suited for our purpose has been investigated in detail both in the laboratory and on a pilot plant scale. A good method, capable of further improvements, has been worked out and this has been tried on the pilot plant involving big batches. On the basis of the experience gained, the department can undertake the production of about 30,000 lbs. of paludrine per year at a cost of about Rs. 20/- per pound. The gain by this can be calculated when we compare this price with that prevailing in the market which is about five times as much. These processes are covered by patents held by the I.C.I. The Government of India are negotiating with the firm for a licence to manufacture the drug. The methods of manufacture of the other important antimalarial, "Chloroquin" are being investigated. This antimalarial appears to be more effective than paludrine for the treatment of clinical attacks, but is more expensive to make. Enough of this drug has been prepared for clinical trials.

The demands for the antiseptic solution, "Pemon", developed in the Department, are increasing because it is very effective and far cheaper than the similar preparations available in the market. The Government

has instructed the Government Hospitals and dispensaries to use this antiseptic. So far, 2000 lbs. of this antiseptic have been supplied and the demand for this is increasing. The department continues to prepare and supply phenyl mercuric nitrate which is being used as a preservative in the vaccines prepared in the Institute.

Research work carried out by the department on the chemotherapy of tuberculosis and malaria and allied researches are described in Part II. A large number of sulphanilamide, sulphone and heterocyclic compounds of various types have been prepared. Many types of quinoline and thiazole derivatives as possible antimalarials have been prepared. Various methods of synthesis of N¹: N⁵-di-substituted diguanides have been investigated to evolve a good process of manufacture of paludrine. An inquiry under the I.R.F.A. is being conducted for studies in the chemotherapy of malaria. The testing of compounds synthesised for antimalarial activity is being handicapped for lack of supplies of young chicks.

The scheme sanctioned by the I.R.F.A. for researches on penicillin continued its work till 31st March. Commercial samples sent for the assay of penicillin are analysed by the department. Work carried on the metabolism of *P. Chrysogenum* are given in Part II.

SHARK LIVER OIL SECTION

This section continued the manufacture of Vitamin A concentrate and processed 19,635 lbs. of shark liver oil; concentrates of an average potency of 250 thousand I. U. per gm. was manufactured during the period under report. A number of experiments were conducted to obtain the best yields in the manufacture of vitamin A and to make the vitamin concentrate as odourless as possible. Experiments were carried out to investigate the possibility of manufacture of vitamin A concentrate from shark liver oil by treatment with different solvents at different temperatures without saponification. The results are sufficiently encouraging to warrant further investigations.

Vitamin A concentrate and Vitamin A-D Tablets manufactured at this Institute were standardised with respect to their vitamin A content by two methods :

- (i) SbCl_3 tintometric method and
- (ii) the fluorophotometric.

The other vitamins were assayed by the usual standard methods.

VITAMIN TABLET SECTION

Experiments in the preparation of Vitamin A-D and Vitamin B-C Tablets were carried out with different compositions and different materials to ascertain the best composition, to achieve palatability and stability of the vitamins contained therein.

Experiments in the manufacture of Vitamin B₁, Vitamin B₂, Vitamin C and Nicotinic Acid Tablets were also carried out. The manufacture of these was undertaken and is still continued in response to the orders from the Government hospitals, dispensaries and medical practitioners. The demand for these tablets is slowly increasing, inspite of the fact that only a few members of the public are at all aware of the availability of these Vitamin Tablets at such low rates. Unfortunately, our products are not advertised to the public and whatever quantity sold to the members of the public, is mainly through transfer of information from individual to individual.

Experiments to establish the best composition for the manufacture of the tablets of sulphathiazole, paludrine hydrochloride, and chloroquin diphosphate, were carried out successfully. The manufacture of sulphathiazole tablets on a large scale has already been undertaken.

The American Multivitamin Tablets, stocked by the Institute were supplied free of charge to different hospitals and municipalities, under instructions from the Government. Different samples of these were assayed and the potency of the vitamins was seen to have gone down considerably. About 18 millions of these tablets were unpacked, from their original packings. Material found unsuitable for human consumption was sorted out and the good tablets were mixed with preservatives and repacked for distribution.

The following number of tablets were produced during the years under review :—

(1) Vitamin A-D tablets	...	...	...	94,39,470
(2) Vitamin B-C tablets	...	...	...	7,89,030
(3) Vitamin B ₁ tablets	...	...	...	1,74,290
(4) Vitamin B ₂ ...	...	...	...	36,900
(5) Vitamin C tablets	...	...	...	1,97,500
(6) Niacin tablets	...	...	...	16,920
(7) Sulphathiazole tablets	...	...	...	2,50,000

IX. Department of Biochemistry

During the years under report the activities of the department, for lack of staff, were chiefly confined to giving useful aid to hospitals and medical practitioners by carrying out the various biochemical examinations necessary for the diagnosis of diseases. Some of the methods used in clinical biochemistry have been reinvestigated with a view to obtain greater accuracy and saving of time and certain improvements have been effected.

1. *Examination of milk samples.*—In order to assist the milk distribution scheme of the Government of Bombay, the routine examination of milk samples collected from the different distribution centres in Bombay was carried out, the total number being 1,532.

2. *Inhibition of growth of plague bacillus.*—Casein hydrolysate prepared by digesting Casein with sulphuric acid is being used in this Institute as a medium for the growth of plague bacillus. A very interesting observation that has been made is that when such a hydrolysate was decolorised with charcoal the rate of growth of plague bacillus was found to be much higher than in the undecolorised medium. Addition of aqueous extracts of charcoal absorbate was found to inhibit the growth of the bacillus to as much as 50 per cent. Some of the amino acids of the medium are probably inhibiting the growth. Further work on this is under progress.

3. *Routine examination.*—The number of samples examined shows a steady increase during the years under report, the total number for the two years being 4,175: Blood 2,703, Urine 346, Cerebro-spinal Fluid 874, Special Tests 252, such as, 71 gastric analysis, 64 blood urea clearances, 55 basal metabolic rate determination, 56 glucose tolerance tests, 1 liver function test, 5 faeces analysis etc. The routine examinations were mostly determinations of non-protein nitrogenous constituents such as urea, uric acid, and serum proteins, calcium, phosphorus, phosphatase, etc.

X. Department of Nutrition and Experimental Pathology.

A. NUTRITION SECTION

As in previous years, the department continued to render useful services to the Civil Supplies Department of the Government of Bombay. During the years under review, 368 different samples of food, and other proprietary preparations were analysed in the department, both on behalf of the Government and the public. These include, estimation of vitamins, e.g. vitamin A, Thiamine, Riboflavin, Nicotinic Acid and Vitamin C (Ascorbic Acid), etc., and proximate principles. Samples such as oil and ghee etc., were also analysed for their acid, saponification and iodine values, and in certain cases, the R. M. value and the Polenske Number were also determined. In several cases food-grains mostly imported from abroad, and similar samples were analysed for the Government with a view to finding out whether they are fit or not for human consumption.

At the instance of the Ministry of Food, Government of India, an extensive investigation was undertaken on the problem of the nutritive value of vanaspati. Experiments were conducted on rats in the laboratory, as well as on human beings in the David Sassoon Industrial School, Matunga, Bombay, to find out the comparative nutritive value of vanaspati and crude groundnut oil, and study the deleterious effect, if any, of feeding vanaspati over a long period.

Under the auspices of the Indian Research Fund Association, the research enquiry which was commenced in the year 1944, on the possible role of nutritional factors in the production of cirrhosis of liver was

continued during these years. The details of the work are given in the Research Section of this report.

During the latter part of the year 1947, Miss Surjeet Chopra, B.Sc., a student from Allahabad Agricultural College, was given training in Nutrition and in the Analysis of Blood, tissues, and Foods, for about three months.

In July, 1947, Dr. M. V. Radhakrishna Rao, Officer-in-charge of the Department of Nutrition and Experimental Pathology, was sent abroad on deputation by the Government of Bombay for higher studies in the field of Nutrition. He visited the leading Universities in the United Kingdom, U. S. A., Canada and Australia and collected first-hand information regarding the recent researches in the field of Nutrition.

B. PATHOLOGY SECTION

During the years under review, 1,554 pathological specimens, received from different hospitals in the province, were examined. They include brains from suspected rabid animals, neoplasms, gynaecological conditions, tuberculosis of different tissues and some rare and interesting pathological specimens. In addition, 572 tissues of experimental animals were examined. These were mostly from the Experimental Pathology Section, and from the other Research Inquiries conducted at the Institute. In all 2,126 specimens were examined in the department.

The table below gives a rough classification of the morbid histological condition of the tissues received and examined at the Institute during the years 1947 and 1948.

Statement showing the number of pathological tissues received and examined at the Institute during the years 1947 and 1948

	1947	1948
I. NEOPLASMS		
<i>(a) Malignant :—</i>		
<i>(1) Carcinoma primary :—</i>		
Adenocarcinoma ...	9	7
Anaplastic carcinoma ...	1	..
Basal-cell Carcinoma ...	1	1
Carcinoma Simplex ...	9	15
Diffuse Carcinoma ...	...	1
Epidermoid Carcinoma ...	1	..

				1947	1948
I. NEOPLASMS— <i>contd.</i>					
(a) <i>Malignant—contd.</i>					
(1) Carcinoma primary— <i>contd.</i>					
Medullary Carcinoma ...	...	...	...	1	1
Mucoid Carcinoma ...	...	...	...	2	...
Papillary Carcinoma ...	...	...	...	4	1
Scirrhus Carcinoma ...	...	...	...	6	..
Squamous-cell Carcinoma	...	...	...	17	15
(2) Carcinoma Secondary ...	...	...	...	7	9
(3) Sarcoma :—					
Fibrosarcoma ...	...	...	...	5	1
Fibromyxosarcoma ...	...	...	...	..	1
Leiomyosarcoma ...	...	...	...	...	1
Myosarcoma ...	...	...	...	...	1
Neurogenic Sarcoma ...	...	...	...	...	1
Osteogenic Sarcoma ...	...	...	...	1	1
Reticular-Cell Sarcoma	...	...	...	..	2
Round-Cell Sarcoma ...	...	...	...	..	2
(4) Malignant lymphoma :—					
Hodgkin's Sarcoma ...	...	...	...	..	2
Lymphosarcoma ...	...	...	...	2	1
(5) Seminoma ...	...	...	...	2	7
(6) Ewing's Tumour ...	...	...	...	1	..
(b) <i>Non-malignant Neoplasms—</i>					
Adamantinoma ...	...	...	...	1	..
Adenoma ...	...	...	...	..	2
Adenomyoma ...	...	...	...	..	5
Cavernous angioma ...	...	...	...	2	..
Chondroma ...	...	...	...	1	..
Colloid goitre ...	...	...	...	..	1
Cyst adenoma ...	...	...	...	3	1
Duct papilloma ...	...	...	...	1	..
Fibroadenoma ...	...	...	...	13	5
Fibroma ...	...	...	...	3	8
Fibrolipoma ...	...	...	...	3	4

					1947	1948
I. NEOPLASMS—<i>contd.</i>						
<i>(b) Non-malignant Neoplasms—contd.</i>						
Fibromyoma	...	...	...	...	23	10
Giant-cell tumour	...	...	...	...	3	..
Granuloma	...	...	...	...	..	2
Granulosa-cell tumour	...	...	...	...	..	1
Haemangioma	...	...	...	...	..	1
Lipoma	...	...	...	...	3	..
Lymphangioma	...	...	...	...	3	..
Melonoma	...	...	...	...	..	1
Mycetoma	...	...	...	...	1	..
Myoma	...	...	...	...	..	1
Myxoma	...	...	...	...	1	..
Neurofibroma	...	...	...	...	2	1
Osteochondroma	...	...	...	...	1	..
Osteoma	...	...	...	...	1	..
Papilloma	...	...	...	...	8	6
Papillary Cystadenoma	...	...	...	...	..	1
Polypus	...	...	...	...	18	26
Pseudomucinous cystadenoma	...	...	...	...	3	1
Submucous fibroid	...	...	...	...	..	1
<i>(c) Mixed parotid tumours</i>	...	...	...	...	6	4
<i>(d) Rhinosporidiosis</i>	...	...	...	...	1	1
<i>(e) Haemangio-endothelioma</i>	...	...	...	...	1	2
<i>(f) Teratoma</i>	...	...	...	...	1	4
II. TUBERCULOSIS.						
Abdomen	...	...	...	...	..	2
Ankle	...	...	...	...	1	..
Appendix	...	...	...	...	..	1
Axilla	...	...	...	...	..	2
Brain	...	...	...	...	1	..
Diaphragm	...	...	...	...	1	..
Elbow	...	...	...	...	..	1
Female Genital tract	...	...	...	...	18	14

Some interesting specimens received during the years 1947 and 1948 were :

Arterio-sclerosis of the radial artery ; Dermoid cyst in the mid-sternal region ; Osteoclastoma of femur ; Lymphangioma of right eyebrow ; Neurofibroma of the palm and axilla ; Rhino-scleroma of nose ; Mycetoma of foot ; Vesicular mole in uterus and Gynecomastism of the male breast.

XI. Department of Virus Diseases including Rabies

This department in its twenty-fifth and twenty-sixth years of existence continued to (a) afford antirabic treatment to patients in the northern part of the Island of Bombay ; (b) prepare very large quantities of antirabic vaccine for issue to antirabic treatment centres ; (c) assist in diagnosis of rabies in animals by histological examination of brain tissues ; (d) hold and conduct yellow fever inoculations with the assistance of the special staff sanctioned by the Government of India and (e) conduct research.

(a) *Antirabic treatment centre at this Institute.*—During the years under report, 9,763 persons attended for antirabic treatment at the Institute. Of these 7,811 completed the full course of treatment, 1404 were advice cases. In cases where there was a doubt about the condition of the dog inflicting the bite and the dog was available for observation the patient was given two injections and was advised to keep the dog under observation for 10 days. At the end of this period of observation, if the dog was healthy, no further treatment was given and the case was classified as an advice case.

(b) *Preparation of antirabic vaccine.*—5% Semple's phenolised sheep brain vaccine was prepared as usual but in very large quantities and issued to various antirabic treatment centres dependent on this Institute for supplies and to indentors from northern India and Pakistan. Antirabic treatments issued by months are shown in the accompanying Table I.

TABLE I.—*Number of Antirabic treatments issued to different antirabic treatment centres during 1947 and 1948 and the bulk supplies made to indentors, not usually dependent on this Institute for supplies of antirabic vaccine.*

Months	Number of treatments issued		Number of treatments for veterinary purposes		Bulk supplies in c.c.	
	Year 1947	Year 1948	Year 1947	Year 1948	Year 1947	Year 1948
January ..	874	865	45	55	—	25,000.
February ..	768	749	48	26	—	11,500
March ..	1,029	841	55	40	—	17,900
April ..	839	1,104	54	29	—	5,230
May ..	1,077	887	54	37	—	2,260
June ..	865	780	45	54	—	1,560
July ..	1,120	934	123	56	—	380
August ..	712	829	48	90	—	Nil
September ..	798	1,022	54	53	75,600	28,000
October ..	910	955	63	40	50,100	7,600
November ..	652	926	33	43	4,400	1,000
December ..	924	749	26	42	32,520	1,500
Total :—	10,568	10,641	648	565	1,62,620	1,01,953

It would be observed that of the 21,209 treatments issued 1,213 were for veterinary purposes. The canine vaccine previously prepared was discontinued as it did not show any immunising value when assayed by Habel's technique.

Bulk supply of 162,620 c.c. was made to the Director-General of Health Services, for northern India, Indian States and other provinces during the year 1947. These supplies were necessitated by disturbances in the north and dislocation of the normal sources of supply.

The following new antirabic treatment centres were opened during the year 1947 :—

- (1) Railway Hospital, Bulsar
- (2) „ „ Gangapur

- (3) Railway Hospital, Bandikui
- (4) „ „ Abu Road
- (5) Ahwa Dispensary, Dang.
- (6) State Hospital, Jobat.
- (7) Raghunath Hospital, Surguja State, Ambikapur
- (8) Jasden State.
- (9) Dewas Senior
- (10) Roberts Hospital, I.P.Mission, Borsad (Dist. Kaira).

The following new antirabic treatment centres were opened during the year 1948 :—

- (1) Municipal Dispensary, Viramgaum
- (2) Burhanpur Hospital (C. P. & Berar)
- (3) Haridas Hospital, Nadiad
- (4) Cantonment General Hospital, Ahmednagar.
- (5) Sheth Vadilal Sarabhai General Hospital and Sheth Chinai Maternity Home, Ahmedabad

The following antirabic treatment centres were abolished during the year 1948 reducing the total to 119.

- (1) Quetta
- (2) Hyderabad (Sind)
- (3) Karachi
- (4) Sikarpur
- (5) Larkana
- (6) Nowabshah
- (7) Dadu
- (8) Jacobabad
- (9) Mirpurkhas
- (10) Sukhur
- (11) Harigaon
- (12) Panchmarhi
- (13) Padma Kuwerha Hospital, Rajkot.
- (14) Bassein

(c) *Antirabic Treatments.*—Detailed information regarding treatments carried out is set out in Table II.

TABLE II.—*Details of treatment carried out at the Institute and the out-station centres in 1944-45-46-47-48 are summarised below.*

Details	1944	1945	1946	1947	1948
(1) Number of persons who applied for treatment ...	4,578	4,853	4,165	4,638	5,125
(2) Advice cases ...	516	481	706	602	802
(3) Fully treated ...	4,031	4,372	3,459	4,036	3,775
(4) Discontinued treatments ...	31	Nil.	Nil.	Nil.	548
(5) Percentage of health returns ...	98·7	97·18	97·19	95·14	Awaited
(6) Cases of hydrophobia ...	1	Nil.	Nil.	Nil.	Nil.
(7) Mortality rate ...	0·02	Nil.	Nil.	Nil.	Nil.
(8) Number of antirabic cetres ...	108	111	117	128	119
(9) Number of treatments sent to the centres ...	8,335	8,947	9,255	10,568	10,641
(10) Number of treatments sent to the Veterinary Surgeons ...	584	649	632	648	565

(d) *Histological examination of brain tissues of animals suspected of rabies.*—Brain tissues examination for presence of Negri-bodies revealed interesting results. These are shown in Tables III, IV and V.

TABLE III.—*Source of brains received and examined for rabies from Bombay City, adjoining suburbs and outstations.*

	Positive		Negative		Unfit		Total		Percentage.	
	1947	1948	1947	1948	1947	1948	1947	1948	1947	1948
Bombay City	31	28	16	19	1	2	48	49	41·74	42·24
Suburbs	7	..	1	1	1	..	9	1	7·83	0·86
Outstations	36	22	17	23	5	21	58	66	50·43	56·9
Total ..	74	50	34	43	7	23	115	116	100·0	100·0

TABLE IV.—*Showing the number of brains received and examined at the Haffkine Institute for evidence of rabies during the years 1947 and 1948.*

Details	Number		Percentage	
	1947	1948	1947	1948
Positive for rabies (Negri bodies present) ..	74	50	64·3	19·18
Negative for rabies (Negri bodies absent) ..	34	43	29·6	37·1
Received in a state unfit for examination ..	7	23	6·1	43·1
Total ..	115	116	100·0	100·0

TABLE V.—*Statement showing the classification of different animals with the results of their brain examination for rabies during the years under report.*

Animals			Positive		Negative		Unfit		Total	
			1947	1948	1947	1948	1947	1948	1947	1948
Dog	..	..	70	44	31	32	6	17	107	93
Monkey	..	..	0	..	1	..	0	..	1	..
Cows and Calf	..	..	1	..	0	2	0	1	1	3
Goat	..	..	2	1	0	1	0	..	2	2
Cat	..	..	1	1	2	2	0	..	3	3
Human	..	..	0	..	0	..	1	..	1	..
Jackal	..	..	..	..	..	1	..	..	..	1
Horse and Pony	..	..	..	1	..	2	..	..	..	3
Bullock	..	..	..	1	..	1	..	4	..	6
She buffalo	..	..	..	2	..	1	..	1	..	4
Rabbit	..	..	..	..	..	1	..	..	..	1
Total	..	..	74	50	34	43	7	23	115	116

(e) *Yellow Fever Vaccine and Vaccination.*—Yellow Fever Vaccine given by Rockefeller Institute in 1946 was tested regularly and was found to have retained its potency fully. Each batch used for inoculation was tested not more than three months previous to its use, and, if found potent, was used.

The Institute distributed yellow fever vaccine out of its stock to all yellow fever inoculation centres in India including Pakistan (1947), Indian States and Portuguese possessions and Ceylon, under instructions from the Director-General of Health Services, India, New Delhi.

Yellow Fever vaccination of air and sea passengers from Bombay proceeding to or passing through yellow fever endemic areas was carried out. 20687 persons were vaccinated during the years under report.

Monthly inoculation against yellow fever during 1947 and 1948 are shown in Table VI.

TABLE VI.

Month					Number of inoculations against yellow fever	
					1947	1948
January	..	..	..	..	950	1,136
February	..	..	..	..	1,191	1,836
March	..	..	..	..	682	932
April	..	..	..	..	350	699
May	..	..	..	..	949	1,061
June	..	..	..	..	1,032	729
July	...	..	..	..	830	679
August	..	..	..	..	427	654
September	..	..	..	..	783	995
October	..	..	..	..	745	475
November	..	..	..	..	834	336
December	..	..	..	..	914	1,468
				Total ..	9,687	11,000

(f) *Assays of Vaccine*.—Following Habel's method of assaying the antirabic vaccine several determinations were made to assay the five per cent. Semple's antirabic vaccine prepared at this Institute for human and veterinary use. In our hands the method worked satisfactorily with Haffkine Institute inbred white mice. All the samples of vaccine examined showed a high degree of immunity which when expressed in terms of M. L. D. protection of vaccine varied from 31,620 to 100,000.

Several brews of a canine vaccine previously prepared in this department were also assayed. This vaccine showed no immunizing properties.

A culture antirabic vaccine prepared by Dr. Veeraraghavan of the Pasteur Institute, Coonoor, was also assayed and found devoid of any immunizing properties.

XII. Blood Bank Department.

During the years under report, the routine work of plasma processing was continued though the demand was less probably due to the availability of the army disposal stock of plasma in the market at a much lower rate than our plasma and many of the bigger hospitals had obtained a good stock of the same directly at disposal stores.

The free distribution of plasma was also less as the free distribution was stopped by the Surgeon-General during the first half of the year 1947 as the stock on hand at this Institute was very low due to the liberal supply of the same during the latter part of the year 1946 at the time of riots in Bombay. The number of voluntary donors has also fallen down. The mobile blood collecting units is visiting the nearby jails at regular intervals to collect blood from the voluntary donors there.

The blood collecting unit at the Indian Red Cross Society has been absorbed under regular Government auspices from 1st March 1948 and is working as a branch of this Institute at the present premises.

A method of screening the blood donors was introduced to guard against the repeated donations by the same donors. Copper sulphate sp. gravity method was used for this purpose. 2,094 donors were screened with this method of whom 399 were rejected.

The Kahn test of all the samples of the blood is being done in this department now. During these years, 2,983 tests were done and out of them 437 were positive.

The blood with high titred haemagglutinins was supplied to the diagnostic reagent department for preparation of blood grouping sera. 11,400 c.c. of blood was supplied for this purpose, during the period under report.

Three batches of doctors attended the course of lectures and demonstrations on blood bank technique etc. arranged by the Surgeon-General.

The following statement shows the progress of work done in this department during the years 1947 and 1948.

TABLE I—*Showing the number of blood donors*

Centre	Type of donors	Number of blood donors	
		1947	1948
Haffkine Institute ..	.. Voluntary ..	249	489
	Paid ..	1,016	611
	Total ..	1,265	1,100
Red Cross Centre ..	.. Voluntary ..	78	46
	Paid ..	1,312	636
	Total ..	1,390	682
Other Hospitals ..	.. Voluntary ..	287	198
	Paid ..		
	Total ..	287	198
Grand Total ..		2,942	1,980

TABLE II—*Showing the blood and plasma yield*

Product						Quantity in litres	
						1947	1948
Blood	..	..	..	..	..	741.580	479.630
Liquid Plasma	..	..	..	..	..	393.875	263.050
Dried Plasma	..	..	..	..	..	426.750	252.500

TABLE III—*Showing the distribution of Plasma.*

Nature				Quantity in millilitres	No. of bottles	Quantity in milli- litres	No. of bottles
				1947		1948	
Liquid	Free	...	...	28.500	114		
	Paid	...	...	10.000	40		
Dried	Free	...	...	62.000	248	25.750	103
	Paid	...	...	209.250	837	227.750	913
Canadian	Free	...	...	25.600	64	280.000	700
Total				326.350	1,303	533.500	1,716

During the years under report, the following work was done and is being pursued in greater detail:—

The effects of repeated blood donations on normal individuals.

Investigation of occurrence of erythroblastosis foetilis and isoimmunization in city hospitals. This work was made possible by the kind gift of Rh testing sera by Sir S. S. Sokhey.

The preliminary paper on the 1st subject was prepared and read at the meeting of the Teaching Pathologists held in this Institute.

XIII. Training of Health Personnel

This Institute plays an important part in dissemination of scientific knowledge by affording facilities to persons desirous of learning different types of techniques either for the prosecution of researches or for routine work in their different spheres of activities. In addition short courses of training of a preliminary nature are given to public health personnel with a view to enable them to carry on their disease control work efficiently. As a rule, most of the workers are from this country; but even persons from other far off countries are attracted to this Institute. The details of important and advanced types of training are mentioned in the department concerned; but the names of persons along with periods of training and the department in which they worked, are given below. In addition, a very large number of workers come to the Institute for casual help, as they are too many, their names are not mentioned in the following table:—

Serial No.	Name.	Designation and Address.	Department in which trained.	Duration of training.
1	Dr. C. H. Lala, M.B.B.S.	Assistant Chief Medical Officer, Jasdan State.	Virus Diseases including Rabies.	10th March to 15th March, 1947.
2	Dr. (Miss) L. S. Samant, M.B.B.S.	C/o Roberts Hospital, I. P. Mission, Borsad, District Kaira.	Virus Diseases including Rabies.	2nd to 8th April, 1947.
3	Dr. (Miss) B. P. Velloz, M.B.B.S.	Assistant Medical Officer, Santacruz Residents' Association Dispensary, Santacruz.	Virus Diseases including Rabies.	15th April to 23rd April, 1947.
4	Mr. S. K. Sobti, M.Sc.	Entomologist to the Public Health Department, C.P.&Berar, Nagpur.	Entomology.	1st May, 1947 to 9th May, 1947.
5	Mr. H. M. Pandya.	Laboratory Assistant, B. J. Medical College, Ahmedabad.	Animal House.	26th May, 1947 to 24th June, 1947.
6	Dr. G. R. Mavalankar.	Medical Officer I/c Anti-Rat Campaign, Poona City Municipality.	Entomology.	11th June, 1947 to 26th June, 1947.
7	Mr. D. C. Katugampola.	Laboratory Assistant, Medical Research Institute, Colombo, Ceylon.	Vaccines and Antitoxins & Sera.	1st July, 1947 to 29th July, 1947.
8	Dr. K. K. Shah, M.B.B.S.	Medical Officer, Ahwa.	Virus Diseases including Rabies.	2nd September, 1947 to 9th September, 1947.
9	Dr. K. N. Neogi.	C/o All India Institute of Hygiene and Public Health, Calcutta.	Antitoxins and Sera, Clinical Pathology and Virus Diseases including Rabies.	25th September, 1947 to 4th October, 1947.
10	Dr. P. S. Unalivia.	Officer-in-charge Serum and Vaccine Production, Institute of Hygiene of the Philippines, Manila.	Antitoxins and Sera and Vaccines	October to December, 1947.
11	Dr. N. P. Parikh, M.B.B.S.	Medical Officer I/c Dispensary, Viramgam, District Ahmedabad.	Virus Diseases including Rabies.	23rd December, 1947 to 30th December, 1947.
12	Mr. T. A. Ramakrishnan, M.Sc.	Chief Chemist, Government Fisheries Laboratory, Travancore.	Chemotherapy.	February, 1948.
13	Dr. C. S. Ramachandra Rao, M.B.B.S., D.P.H.	Provincial Blood Transfusion Officer, Madras.	Blood Bank.	5th July, 1948 to 10th July, 1948.
14	Mr. S. V. Ponnambalam.	Senior Laboratory Assistant, Medical Research Institute, Colombo, Ceylon.	Antitoxins and Sera and Vaccines.	11th August, 1948 to 30th August, 1948.
15	Captain D. M. Gomez, IAMC.	Clinical Department, A.F.M.C.	Blood Bank.	26th August, 1948 to 15th September, 1948.
16	Mr. S. S. Bhatt, B.Sc.	Technical Assistant, Public Health Laboratory, Surat.	Clinical Pathology and Bio-chemistry.	8th October, 1948 to 21st October, 1948.
17	Dr. Ghulam Ahmed.	Officer I/c Plague Operations, Hyderabad-Deccan.	Entomology.	15th December, 1948 to 25th December, 1948.

XIV. Critics Club

During the years 1947 and 1948, 43 and 49 meetings were held respectively at which, abstracts of current literature pertaining to various subjects were read by the members of different departments, in addition to the researches reported below. Farewell functions to the retiring members, Mr. M. A. Malandkar and Dr. S. P. De were held on 17th January, 1947 and 24th September, 1948, respectively. The members of the Critics Club and other staff of the Institute recorded their deep sense of sorrow at the premature death of Lady Leila Sokhey and offered their heart-felt condolence to Major-General Sir Sahib Singh Sokhey.

Members of the Critics Club had two interesting lectures from the distinguished visitors, Dr. D. G. Davey of the Imperial Chemical Industries and Dr. Gellner of the World Health Organisation, who spoke on "Paludrine" and "B. C. G. Vaccine" respectively. Original work and reviews were reported on the following subjects :—

1. "A study of Weil-Felix reaction in the diagnosis of typhus in Bombay" by Dr. D. W. Soman on 31st January 1947.
2. "Protein deficiency in man" by Mr. N. C. Dutta on 28th February 1947.
3. "Sulphamerazine and Sulphadiazine in the treatment of bubonic plague" by Dr. P. M. Wagle on 30th May 1947.
4. "Blood donors in India" by Dr. K. V. Kulkarni on 4th July 1947.
5. "Importance of choline in nutrition" by Mr. N. C. Dutta on 25th July 1947.
6. "Recent advances in chemotherapy of malaria" by Dr. K. Ganapathi on 29th August 1947.
7. "Factors influencing the time of flocculation (Kf) in Ramon's flocculation test for diphtheria" by Dr. S. P. De on 26th September 1947.
8. "Bacteriological types of *C. diphtheria* met with in Bombay" by Dr. D. W. Soman on 31st October 1947.
9. "Plasma proteins in health and disease with special reference to Cirrhosis" by Mr. N. C. Dutta on 30th January 1948.
10. "An outbreak of cholera in Bombay with reference to its serological types" by Mr. S. K. Nail on 27th February 1948.
11. "Lathyrism" by Mr. N. C. Dutta on 30th April 1948.
12. "Standardisation of antivenene" by Mr. Nimbkar on 28th May 1948.

13. "Pyrogens" by Mr. Bharucha on 27th August 1948.
14. "Philosophy of Science" by Dr. K. Ganapathi on 29th October 1948.
15. "Yellow Fever Vaccine" by Dr. Parakh on 26th November 1948.
16. "Typhus Fever in Bombay and its latest treatment" by Dr. D. W. Soman on 31st December 1948.

Two meetings of the Teaching Pathologists were held at the Haffkine Institute and the following papers were read and discussed.

1. "Scrub typhus in Bombay" by Dr. D. W. Soman on 22nd February 1947.
2. "Preparation of casein-hydrolysate for laboratory and therapeutic use" by Mr. K. H. Bharucha on 22nd February 1947.
3. "Assay of Penicillin" by Mr. M. R. Radhakrishnan on 28th February 1948.
4. "Factors influencing the time of flocculation in Ramon's flocculation test for diphtheria" by Dr. S. P. De on 28th February 1948.
5. "Blood donor problem in Bombay" By Dr. K. V. Kulkarni on 28th February 1948.

XV. Income and Expenditure

The following tables show the income and expenditure of the Haffkine Institute for the two years under review :—

1946-47

Receipts		Expenditure	
	Rs.		Rs.
XXVII—Medical—		38—Medical—	
Sale proceeds of Sera, Vaccines and miscellaneous receipts	14,23,036	H—Bacteriological Laboratories—	
		H-1 Haffkine Institute,	
		H-2 Pasteur Institute,	
		F—Special Development Programme—	
		F-3 Scheme for serum manufacture—	
		Pay of Officers ...	1,83,876
		Pay of Establishment ...	2,09,408
		Allowances etc. ...	2,08,527
		Contingencies. ...	10,62,018
Total Receipts ...	14 23,036	Total expenditure ...	16,63,829

1947-48

Receipts		Expenditure	
	Rs.		Rs.
XXVII—Medical—		38—Medical—	
Sale Proceeds of Sera, Vaccines and Miscellaneous Receipts	30,06,656	H-Bacteriological Laboratories— H-1 Haffkine Institute,	
		H-2 Pasteur Institute,	
		Pay of Officers	... 1,78,214
		Pay of Establish- ment ...	... 2,57,052
		Allowances etc.	... 2,22,492
		Contingencies	12,06,489
Total Receipts	... 30,06,656	Total expenditure.	18,64,247

PART II

RESEARCH WORK

I. Sulphonamides and Antibiotics in the Treatment of Plague. Experimental Infection

(SOKHEY AND HABBU)

The evaluation of therapeutic agents in the laboratory in plague is a comparatively easy matter. As plague is essentially a disease of rodents and only incidentally spreads to human beings, it is possible to reproduce the natural disease in experimental animals. We have worked out a laboratory test for the purpose and have fully described the experimental procedure elsewhere. We use Haffkine Institute imbred white mouse weighing 21—30 g. as the test animal. This animal is highly susceptible to plague; subcutaneous injection of ten virulent organisms give a 100 per cent mortality. For experimental infective dose we give 75-150 organisms. The animals develop septicaemia 48 to 72 hours after infection, and 100 per cent die within 4 to 8 days. Selected strains of the plague bacillus are preserved by drying from the frozen state. In this form, they maintain their virulence undiminished almost indefinitely; we have now used the same dried culture for over seven years.

With the availability of such a laboratory test, we have always tested out the given therapeutic agent in experimental infection before making clinical trials. In our studies extending over several years the results of the tests in experimental infection have always paralleled those of clinical trials.

We give the results of trial of sulphadiazine, sulphamerazine and streptomycin in experimental infection.

In human bubonic plague, the most important factor which decides the issue is the development of septicaemia. In mice, under the experimental condition indicated above, septicaemia develops from 48 hours to 72 hours after the induction of infection by the subcutaneous route. By 48 hours about 50 per cent and by 72 hours 100 per cent of the mice develop septicaemia. In experimental infection, we therefore, start treatment with the therapeutic agent under trial in one batch of animals 48 hours and in another batch 72 hours after the induction of infection. It may be added here for record that if the treatment with these sulphonamides or streptomycin is commenced at the same time as the infection is induced, 100 per cent of the mice recover.

For each test described in this paper, 20-22 animals were infected, the selected dose of the drug under test was given to 10 mice, the remaining 10-12 mice were kept as controls. During the experiment, the mice were kept in batches of 5 in special cages designed for experimental work in tropical countries. The cages were housed in an air-conditioned room with a temperature of 75°-78° F.

The mice were observed for at least 31 days. They were examined once a day and deaths were noted. Those dying within this period were dissected and examined for evidence of plague. The surviving animals were similarly examined.

The sulphonamides under test, i.e. sulphadiazine, sulphamerazine and sulphamethazine, were made into an emulsion with 10 per cent. solution of gum acacia so that 0.5 ml. of the emulsion contained the selected dose. This quantity of the emulsion was introduced with a pipette into the stomach of the mouse. The drugs were administered in a dose of 5 mg. four times a day at 11 a.m., 5 p.m., 11 p.m. and 5 a.m. for five days. The administration of the drugs for a longer period made no difference.

Penicillin and streptomycin were administered subcutaneously, the selected dose being contained in 0.2 ml. of the solution. Penicillin was administered in a dose of 1000 units four times a day for 7 days and Streptomycin in a dose of 0.8 mg. four times a day for the first two days and 0.2 mg. four times a day for the next five days. In the selection of the dose of streptomycin we were helped by the work reported before,

Results

Though several experiments were made, results of only representative ones are given below in Tables I and II.

Table I.—Results of treatment in experimental plague in mice with sulphadiazine. Each drug was given by mouth in a dose of 5 mg. four times a day for five days.

	Period elapsing between infection and drug administration	Number of organisms used in test infective dose	Mice used.	Deaths in mice up to 31 days after infection	Controls Mice used	Deaths
Sulphadiazine ...	48 hours	144	10	5	12	12 (5.7)*
Sulphamerazine ...	48 hours	144	10	3	12	12 (5.7)
Sulphamethazine ...	48 hours	144	10	3	12	12 (5.7)
Sulphadiazine ...	72 hours	144	10	6	12	12 (5.7)
Sulphamerazine ...	72 hours	144	10	3	12	12 (5.7)
Sulphamethazine ...	72 hours	144	10	3	12	12 (5.7)

Table II.—Results of treatment of experimental plague in mice with penicillin and streptomycin. Dose of penicillin, 1000 units four times a day for 7 days. Dose of streptomycin 0.8 mg. four times a day for two days, and then 0.2 mg. four times a day for the next five days.

Penicillin ...	48 hours	142	10	10	10	10 (5.5)*
Streptomycin ...	48 hours	142	10	0	10	10 (5.5)
Streptomycin ...	72 hours	124	10	1	10	10 (6.0)

*The figure in brackets in the last column denotes the average duration of life of the controls,

Though results of the administration of the selected sulphonamides in a dose of 5 mg. four times a day are given in the table, we have also tried doses of 2·5 mg. and 10 mg. twice a day and 10 mg. four times a day for periods varying from 3 days to 10 days. The dose of 5 mg. four times a day for five days appears to give the best results. Both sulphadiazine and sulphamerazine in the larger dose seem to be toxic to mice. The concentration of the drugs in the blood achieved with this dose will be described in another paper.

These experiments show that both streptomycin and sulphonamides have a very remarkable curative action in plague, but the action of streptomycin is definitely superior to those of sulphonamides.

II. Sulphonamides and Antibiotics in the Treatment of Plague Clinical Trials

(WAGLE AND SOKHEY)

During the years under review, two opportunities presented themselves to test in the field the comparative curative value of sulphamerazine, sulphadiazine and streptomycin in the treatment of Bubonic Plague. The first field trial was conducted at Poona from the beginning of March to 25th April 1947. A research unit with the necessary laboratory equipment was sent from the Haffkine Institute. The cost of the research unit was borne by the Indian Research Fund Association—during this trial as well as the subsequent trial. In all 107 cases were treated, of which 100 turned out to be cases of plague. The trial was utilised to determine the comparative curative value of sulphadiazine and sulphamerazine in the treatment of Bubonic Plague.

The second field trial was conducted at Manchar in Poona District in a mobile plague hospital and lasted from 26th November 1947 to 22nd March 1948. During this trial 313 cases were admitted, of which 287 turned out to be cases of plague. This trial was utilised to compare the curative value of streptomycin with that of the sulpha drugs, sulphadiazine and sulphamerazine.

Methods

Diagnosis.—On admission 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 time of admission and its severity, if present. The bubo was also punctured at the same time with a syringe and the fluid drawn planted on agar slopes to confirm the diagnosis. These slopes were incubated at room temperature for two days. If 0·25 c.c. of blood gave less than 10 colonies, it was noted as a case of mild septicaemia,

while cases with over 10 colonies in 0.25 c.c. of blood were considered as cases of severe septicaemia.

During the field trials, it has been noticed that the most important single factor which decides the issue in human bubonic plague is the development and degree of septicaemia. If the lymph gland prevents the spread of infection to blood stream and the infection remains localised, spontaneous recovery results in most of the cases. On the other hand, if the organisms invade the blood-stream and septicaemia results, death almost invariably follows, unless an effective curative agent is given to control the infection. Thus, a truer picture of the results of given treatment is obtained if only those cases are considered which have septicaemia at the time the treatment is started. For this reason, results of each trial are given in two tables. One table shows the results of treatment in all cases and the other of cases with plague septicaemia at the commencement of the treatment.

Division of Cases.—Different treatments were given in strict rotation, no selection of cases was made. In spite of this, a number of cases under different treatments had to be excluded from account for various reasons. Some patients after the commencement of treatment proved not to be cases of plague, some were convalescent on admission. Cases moribund at the time of admission and dying within 24 hours were kept out of account as we found that therapeutic concentration of the sulphonamides in blood was not reached till 4-8 hours after the injection of the drugs and consequently these drugs were not effective within such a short period. An occasional case left the hospital against advice while some received combined treatment.

Dosage and Concentration in Blood

Sulphamerazine.—An initial dose of 4 gms. was followed by 1 gm. 4 hours later. Then 1 gm. was given every 8 hours till the temperature remained normal for 2 days. This dose maintained a concentration between 10 to 20 mgms. per 100 ml. of blood.

Sulphadiazine.—An initial dose of 4 gms. was followed by 2 gms. 4 hours later. Thereafter 1 gm. was given every 4 hours until the patients' temperature remained normal for 2 days. This dose maintained a concentration of between 10-20 mgms. per 100 ml. of blood.

Streptomycin.—In relatively mild cases, an initial dose of $\frac{2}{3}$ gm. followed by $\frac{1}{3}$ gm. every 4 hours was given until the temperature remained normal for 24 hours. In severe cases, $\frac{2}{3}$ gm. of the drug every 4 hours was given until the temperature remained normal for 2-3 days.

In a few cases in which the concentration of the antibiotic in the blood was estimated it was found that concentration reached 5 units in 4 hours, 10 units in 8 hours and 20 units or more after 24 hours per ml. of serum.

Results

Field Trial at Poona

(March 1, 1947 to April 25, 1947)

Total cases of plague treated	...	...	100
Excluded from reckoning—			
Cases moribund at the time of admission and died			
within 24 hours of admission	...	...	12
Cases treated with sulphamerazine cum serum	...	...	1
Cases left hospital while still febrile	...	...	1
Cases convalescent at the time of admission	...	...	5
			19

Cases considered	...	...	81

Treatment and Dosage.—The trial was utilised to determine the comparative curative value of sulphadiazine and sulphamerazine in the treatment of Bubonic Plague. Cases were treated in strict rotation with the different drugs.

The sulphadiazine patients received 10·0 gm. on the first day and 6·0 gm. per day on subsequent days. The blood concentration was between 10-20 mgm. per cent.

Up to case No. 43, sulphamerazine was given in a dosage of 8·0 gm. on the first day and 3·0 gm. per day on subsequent days. From case No. 45, it was given in a dosage of 7·0 gm. on first day and 3·0 gm. per day on subsequent days. Both the dosages maintained a concentration between 10 to 20 mgm. per 100 c.c. of blood.

All cases

Treatment	Number of cases	Number of deaths	Case mortality per cent.
Sulphadiazine ..	40	4	10·0
Sulphamerazine ..	41	3	7·3

Cases with Plague Septicaemia at the Commencement of Treatment

Treatment	Number of cases	Number of deaths	Case mortality per cent.
Sulphadiazine	7	2	28·5
Sulphamerazine	9	2	22·2

Field Trial at Manchar

(November 26, 1947 to March 22, 1948)

Total cases of plague treated	...	...	287
Excluded from reckoning—			
Cases moribund at the time of admission and dying within 24 hours	...	...	14
Cases treated with streptomycin and sulphamerazine or serum	...	...	4
Cases convalescent at the time of admission	...	18	
			36
Cases considered	...		251

Treatment and Dosage.—The trial was utilised to assess the curative value of streptomycin in Bubonic Plague as compared to that of sulphadiazine and sulphamerazine. Cases were treated in strict rotation with different drugs. From case No. 1 to 112 cases were treated alternately with sulphadiazine and streptomycin and from case 113 to 300, they were treated alternately with sulphamerazine and streptomycin. From 301 to 313, however, all cases were treated with streptomycin.

Streptomycin was given in a daily dosage of 2·33 gm. in mild cases and of 4·0 gm. in severe cases. In few cases in which the estimations were done the concentration of the drug was found to be over 10 units in 8 hours and over 20 units after 24 hours per ml. of serum.

All cases

Treatment				Number of cases	Deaths	Case mortality per cent.
Streptomycin	..	..	..	132	5	3·8
Sulphadiazine	..	..	..	47	2	4·2
Sulphamerazine		..	..	72	6	8·3

Cases with Plague Septicaemia at the Commencement of Treatment

Treatment				Number of cases	Deaths	Case mortality per cent.
Streptomycin	..	..	..	32	3	9·3
Sulphadiazine	..	..	..	11	2	18·1
Sulphamerazine		..	..	13	5	38·4

The results obtained with different treatments in this trial may not show statistically significant differences but the effect of streptomycin was very striking. Both in the non-septicaemic as well as in septicaemic cases, the temperature in cases treated with streptomycin came to normal earlier than that in cases treated with sulphadiazine or sulphamerazine. Thus in septicaemic cases treated with streptomycin temperature became normal on an average in 50 hours, with sulphadiazine in 85 hours and with sulphamerazine in 89 hours.

No serious toxic symptoms were noticed with any of the drugs. Two cases treated with streptomycin developed mild temporary psychosis, which disappeared with discontinuance of drug. One case treated with sulphamerazine developed mild dermatitis. No other symptoms were noticed.

The Manchar trial was made possible by the generous gifts of streptomycin by the Medical Research Council of Great Britain and from Messrs. Abbott Laboratories, North Chicago.

Besides the above two trials, the sudden outbreak of plague in Calcutta in April 1948, necessitated the sending of a treatment unit there to carry out the treatment of plague cases. The unit was, however, sent to create confidence in public mind regarding availability of effective treatment and not to taste the value of any drug. Two drugs were used in this trial, streptomycin and sulphadiazine. In order to create confidence in the mind of the public, cases were not treated alternately with the two drugs, but instead, serious cases were treated with streptomycin and mild cases with sulphadiazine. The trial lasted from 1st May 1948 to 19th June 1948, and during this period 44 cases were admitted for treatment, of which 34 turned out to be plague. The drugs were given in the same dosage as in the above two trials.

Of the 34 plague cases, 6 cases are excluded from reckoning, as 2 were convalescent on admission and 4 moribund. Results in remaining 28 cases were :—

All cases

Treatment				Number of cases	Number of deaths	Case mortality per cent.
Streptomycin	..	..	..	16	1	6.25
Sulphadiazine	..	..	..	12	0	0.0

Cases with Plague Septicaemia at the Commencement of Treatment

Treatment				Number of Cases	Number of deaths	Case mortality per cent.
Streptomycin	..	..	..	5	1	20.0
Sulphadiazine	..	..	..	1	0	0.0

III. Sodium Phthalyl Sulphacetamide in the Treatment of Cholera Cases (WAGLE)

As desired by the Honourable Minister for Health, the drug Sodium Phthalyl Sulphacetamide was tried in cholera cases at Erandole, where a mobile hospital was working under the directions of the Director of Public Health. The treatment was given by the Medical Officers of the mobile hospital. A unit was sent from the Haffkine Institute to aid in making a bacteriological diagnosis of cases and to study the absorption of the drug and its toxicity.

The trial lasted from 8th August 1948 to 2nd October 1948 and during this period 50 cases were admitted to hospital, of which 32 proved bacteriologically to be cases of cholera. As it was feared that sufficient number of cases may not be available for treatment with the new drug if alternate cases were kept as controls, as many cases as possible were treated with the new drug, except when at one time during the period of trial the supply of Sodium Phthalyl Sulphacetamide fell short, the cases were treated with Sulphaguanidine. Thus, out of 50 cases admitted to hospital, 37 cases received treatment with Sodium Phthalyl Sulphacetamide, 10 cases received Sulphaguanidine and 3 cases combined treatment with both the drugs.

Dosage.—As no definite information was available as to the optimum dosage of Sodium Phthalyl Sulphacetamide in cholera cases, a daily dosage of 10 gm. was decided upon and given in 5 equally divided doses. Sulphaguanidine was given in a daily dosage of 12 gm. divided in six equal doses.

Absorption of drug in blood and its toxicity.—As sulpha drugs acting locally in the intestines are not expected to be absorbed in the blood to any appreciable degree, concentration of the drug in the blood was estimated once daily during the period of administration of the drug to see to what extent the drug is absorbed in the blood. Blood examination was also done to see if the drug exercised any serious toxic action on the haemopoietic system. These examinations showed that the drug Sodium Phthalyl Sulphacetamide was not absorbed to any appreciable extent in the blood, nor had it any serious toxic effect on the haemopoietic system. No serious toxic symptoms were also noticed in any of the cases treated.

The results obtained in 32 bacteriologically proved cases of cholera are given below :—

Treatment	Number of Cases	Number of deaths	Mortality per cent.
Sodium Phthalyl Sulphacetamide ..	22	4	18.18
Sulphaguanidine ..	8	2	25.0
Sodium Phthalyl Sulphacetamide and Sulphaguanidine ...	2	0	0.0

These results do not show any statistical difference between the two treatments.

IV. Titration of Anti-human Serum

(D. C. LAHIRI)

Preparation of anti-human horse serum, which seems to prevent serum reactions in man and is, therefore, added routinely to all therapeutic sera issued from the Institute, suffered in the past owing to lack of methods of titration of the serum. In the absence of any test for checking the immune response of a horse, the horses had to be bled blindly without knowing whether they had responded to the injections of human plasma at all, or not. Now at least an indirect test is available to determine the degree of response of the horses during immunization.

The test depends upon agglutination of washed human red blood corpuscles by the anti-human horse serum in the presence of synergists like irradiated albumin, but only to a low titre. With the progress of immunization of the horses with pooled human plasma, however, the titre progressively increases. If, therefore, the serum of a horse under immunization fails to show any rise in agglutinating titre, the horse may be discarded as useless for the production of anti-human serum. It is, however, not known whether there is any correlation between the agglutinating titre of an antihuman serum and its anti-allergic activity.

The titre of an antihuman serum shows its maximum against O group cells and its minimum against A group cells. For this reason and also for that the pooled human plasma, which is injected into the horses, is carefully freed from red blood corpuscles, it appears that the agglutinating antibody in the antihuman serum is produced by some non-specific stimulus. It is possible that the stimulus of some heterophilic antigen, which is contained in the normal human plasma, produces the agglutinating antibody in the antihuman serum.

V. Use of Tween-80 for Improvement of Solubility of Lyophilised Serum

(D. C. LAHIRI)

Tween-80, which is a polyoxyalkylene derivative of sorbitan mono-oleate is a non-polar "wetting agent". Due to its non-polarity it does not precipitate proteins in solution. Hence Tween-80 can be used to improve the solubility of concentrated and purified serum after drying from the frozen state. It has been reported by me before that bile salts do improve the solubility of a lyophilised serum, which is otherwise insoluble. Since the properties of Tween-80 were now known, it suggested itself as an alternative.

In a concentration same as that of bile salts, that is 0.1 per cent. Tween-80 was found to improve the solubility of lyophilised sera. But in a sad contrast against the crystal clear appearance of a serum which was treated with bile salts and ether extraction, the serum containing Tween-80 looked cloudy, more cloudy after reconstitution of the lyophilised serum than before drying. This was so because the lipids of the serum were still there in an emulsoid form in the presence of Tween-80. If such a cloudy serum were shaken up with ether, the ether removed Tween-80 in preference to the lipids, and the serum after drying was much more insoluble than before ether extraction. In this respect, therefore, the bile salts proved definitely superior to Tween-80. Because, bile salts facilitated removal of lipids from a serum, in addition to being a "wetting agent" itself, and hence the serum always presented a crystal-clear appearance.

VI. Use of Flocculation Test for Preliminary Assay of Anti-snake Venom Serum

(NIMBKAR AND HAZRA)

For routine assays of the Haffkine Institute polyvalent anti-snake-venom serum the mouse protection test is used, both for preliminary and final assay. The advantages of the use of flocculation test for preliminary assays were, however, so obvious that the question of suitability of the test was opened up again. Past publications report conflicting experiences but this may be due to large numbers of variables involved in the venom-antivenom reaction. We, on our part, have attempted to smoothen the major variable, the venom, by mixing freeze-dried venoms of over 200 snakes of each species to form one sample. Moreover, we have extended the studies, which were limited in the past to only 2 antivenenes anti-cobra and anti-Russell's viper serums, to anti-krait and anti-saw scaled viper serums too. In addition, we have always compared the value of a serum obtained by the flocculation test with that obtained by the mouse protection test.

The flocculation was done by 2 different procedures, (1) constant antibody optimum or α procedure and (2) constant antigen optimum or

β procedure. The α procedure frequently showed 2 zones of flocculation, one of the optima of which closely corresponded with the mouse protection value of the serum. The other zone was called, therefore, as "pseudo zone" to differentiate it from the zone of truly specific flocculation. The β procedure, however, always showed only one zone of flocculation, but the optimum did not always correspond either with the mouse protection value or with α procedure optimum of the truly specific zone of flocculation. In such cases, the β procedure optimum corresponded rather with the optimum of the "pseudo zone" of the α procedure.

That the optimum of the true zone of flocculation of the β procedure, and that of α procedure when it corresponded with the former, represented fully neutralized mixture of the venom and the antivenene, was proved by animal experiments. The supernatants of the optimum flocculated mixtures in such cases, showed excess neither of venom nor of antivenene. On the other hand, optimas of the "pseudo zone" of the β -procedure and of the α procedure when it corresponded with β the "pseudo zone" always showed considerable excess of antivenene in the supernatants of the optimum flocculated mixtures.

Bearing in mind the abovementioned limitations inherent in the flocculation test, it still seems likely that the flocculation test could be profitably used for preliminary assays of anti-snake-venom serum.

VII. On the Pulicidal Effect of DDT

(SHARIF)

Both the jar and burrow experiments with the delousing powder 10 per cent. DDT in pyrophyllite, U. S. Patent No. 2329674, as reported in the previous report, were continued. Experiments with aqueous suspension containing 50 per cent. DDT, prepared by Messrs. Geigy Insecticides Ltd., were also continued.

In the jar experiments a concentration of 1 in 10,000 continued to kill 100 per cent. rat-fleas up to 992 days tried after dusting with the DDT powder. In the artificial W-shaped rat-burrow, when dusted with 60 g. of 10 per cent. DDT powder with a cyanogas "A" foot pump duster, about 100 per cent. ratfleas were killed after 998 days up to a distance of $9\frac{1}{2}$ feet. The percentage of mortality beyond this distance varied from 33 at a distance of about $19\frac{1}{2}$ feet to 75 at a distance of about $11\frac{3}{4}$ feet. Thus the deterioration of DDT owing to volatilization in the burrows as well as in the jars at the room temperature of Bombay is not appreciable even after a period of about $2\frac{3}{4}$ years.

Experiments with aqueous suspension show that 0.297 mg. of DDT per square cm. continued killing 100 per cent fleas up to 579 days tried after spraying with the aqueous suspension of DDT.

VIII. On the Pulicidal Effect of "Gammexane"

(SHARIF)

Gammexane Smoke Generators, No. 2-2 oz. unit-containing the smoke generating mixture with about 3 per cent. of Gammexane, prepared by the Imperial Chemical Industries (India) Ltd. were used for ascertaining their pulicidal value in the artificial W-shaped rat-burrows. When smoked with one such generator, about 100 per cent. rat-fleas, *Xenopsylla cheopis*, were killed in an experiment tried after 45 days of smoking the burrow at a distance of 20 feet and 4 inches from the entrance.

A little smoke escaped from the farthest end of about twentyone feet long artificial rat-burrow. Distribution of the smoke was unequal, being very great in the first arm. Most of the smoke settled at the bottom of the burrow. The smoke was not poisonous to the domestic rats, when kept in the burrow for twenty-four hours.

IX. On the Rodenticidal Effects of Dethdiet Red Squill and Rodine

(SHARIF)

Experiments to test the rat poisons, Dethdiet Red Squill and Rodine, manufactured by S. B. Penick & Co., New York, were carried out. For comparison barium carbonate was used. The poisons were given in the form of baits of 1 g. each. One part of any of the first two poisons with 9 parts of the base, maize flour, was equally effective for *Rattus rattus rufescens*, *Rattus norvegicus* and *Gunomys kok* as 1 part of barium carbonate with 4 parts of the base, there being no statistical difference in the mortality rates with the three poisons.

Poison used	R.r. rufescens		R. norvegicus		G. kok	
	No.	died	No.	died	No.	died
Dethdiet Red Squill	23	18	6	6	20	19
Rodine	23	21	6	6	20	19
Barium carbonate	18	17	6	4	10	10

In most cases the acceptability of baits containing Dethdiet Red Squill or Rodine was equally good as of those containing barium carbonate; but in a few cases it was significantly better in the former case.

One serious defect was, however, found with the Dethdiet Red Squill that it not only caked; but during rainy months, when kept open, it absorbed moisture to such an extent that it became soggy.

X. Synthesis of Asymmetrical Diaryl Sulphones and Aryl-quinolyl Sulphones

(GANAPATHI AND BELLARE)

Substituted phenyl-sulphinic-acids were allowed to interact with benzoquinone and substituted benzoquinones in aqueous alcohol followed by heating on waterbath to obtain dihydroxy-diaryl-sulphones, according to the method of Hinsberg. Thus the condensation of p-acetaminobenzenesulfinic acid with 2:5-dichlorobenzoquinone in aqueous alcohol afforded p-acetaminophenyl-2':5'-dihydroxy-3':6'-dichlorophenyl sulphone.

A number of substituted phenylsulphinic acids like p-acetamino, p-chloro-, p-bromo-, 2:5-dichloro-, and p-acetaminomethyl, etc. -phenyl sulphinic acids were condensed with substituted benzoquinones like 2-chloro-, 2-bromo-, 2:5-dichloro-, etc. -benzoquinones, obtaining corresponding sulphones.

In the aryl-quinolyl series a number of substituted quinolines containing a halogen in either 2 or 4 position were refluxed in either ethyl alcohol or in some cases higher alcohols, with substituted phenyl sulphinic acids in the form of their alkali-metal salts or in presence of potassium-acetate. In some cases it was necessary to add a trace of copper powder and iodine as catalysts. Thus the condensation of 4:7-dichloroquinoline with 2:5-dichlorobenzenesulphinic acid was achieved by refluxing them together with potassium acetate in ethyl alcohol for about two hours, yielding 4-(2':5'-dichlorophenyl sulphonyl)-7-chloroquinoline.

A number of substituted quinolines like 4:7-dichloro-, 2-methyl-, 4:6-dichloro-, 2-methyl-4-chloro-6-methoxy-, 2-chloro-4:6-dimethyl-, etc. -quinolines were condensed with various substituted phenyl-sulphinic acids like p-acetamino-, p-acetaminomethyl-, p-chloro-, p-bromo-, 2:5-dichloro-phenyl sulphinic acids yielding corresponding sulphones.

XI. Synthesis of Some Sulphanilamide and Sulphone Derivatives of Thiazole

(GANAPATHI AND MRS. VENKATARAMAN)

Condensation of 2-bromo-5-nitrothiazole with sulphanilamide yielded (i) in the presence of potassium carbonate and copper powder, 2-sulphanilamido-5-nitrothiazole and (ii) in alcoholic solution the isomeric N⁴- (5-nitro-2-thiazolyl)-sulphanilamide. 2-Hydroxy-4-methyl-5-aminothiazole and 2:4-dimethyl-5-aminothiazole condensed with acetsulphanilylchloride to yield respectively 2-hydroxy-4-methyl-5-acetsulphanilamidothiazole and 2:4-dimethyl-5-acetsulphanilamidothiazole. The latter, on hydrolysis, furnished 2:4-dimethyl-5-sulphanilamidothiazole, which was about as active as sulphathiazole in experimental pneumococcal and *P. pestis* infections in mice but much more toxic. By condensing 2-methylthiazole, 2:4-dimethyl-

thiazole and 2-hydroxy-4-methylthiazole with formalin and sulphanilamide, were obtained respectively 2- β -(p-aminobenzenesulphonamido) ethylthiazole, 4-methyl-2- β -(p-aminobenzenesulphonamido) ethylthiazole and 2-hydroxy-4-methyl-5-(p-aminobenzenesulphonamido) methylthiazole, none of which showed any therapeutic activity in experimental pneumococcal infections in mice. Condensation of potassium acetaminobenzenesulphinic acid with 2-chloro-4:5-dimethylthiazole, 2-chloro-5-ethylthiazole and 2-bromo-5-nitrothiazole, the corresponding 2-p-acetaminobenzene sulphonylthiazoles were obtained which were deacetylated to the corresponding amino compounds; these compounds also did not show striking therapeutic activity.

XII. A Study of the Methods of Synthesis of Diguanydes

(FERNANDES AND GANAPATHI)

In the course of attempts to prepare quinoline derivatives with substituted diguanide side chains, the methods of syntheses of N¹-p-chlorophenyl-N⁵-isopropylidiguanide (I) as a model compound have been investigated. The diguanide (I) was obtained by the condensation of isopropylguanidine with (1) p-chlorophenylcyanamide, (2) S-methyl-p-chlorophenylisothiouric acid and (3) p-chlorophenylthiourea in the presence of lead acetate. Attempts to prepare the compound (I) by the condensation of p-chlorophenylisothiocyanate with isopropyl guanidine were unsuccessful. p-Chlorophenylisothiocyanate condensed with S-methyl isothiouric acid to yield S-methyl-N¹-(p-chloroanilino)-thioformylisothiouric acid (II) but the methylmercapto group of this compound could not be replaced by either imino or isopropylimino groups. p-Chloroaniline condensed with isopersulphocyanic acid to yield p-chlorophenyldithiobiuret which on boiling with ammonia and isopropylamine in the presence of mercuric oxide furnished respectively p-chlorophenyldicyandiamide and N-p-chlorophenyl-N'-isopropyl-N''-cyanoguanidine (III). p-Chlorophenyldithiobiuret on oxidation with iodine yielded p-chlorophenylthiurea hydroiodide which on treatment with ammonia and isopropylamine in the presence of mercuric oxide furnished respectively p-chlorophenyldicyandiamide and N-p-chlorophenyl-N'-isopropyl-N''-cyanoguanidine. On treatment with isopropylamine, p-chlorophenylthiourea yielded only N-(p-chlorophenyl)-N'-isopropyl-N''-aminothioformylguanidine, which easily undergoes desulphurisation to yield the compound (III): however, in the presence of alcoholic ammonia and mercuric oxide, p-chlorophenyldicyandiamide was obtained.

XIII. Synthesis of possible Lipophilic Chemotherapeutics of the Sulphanilamide and Sulphone Series derived from Fatty Acids, including those of the Chaulmoogra Group

(DELIWALA, RAJAGOPALAN AND GANAPATHI)

Twentythree compounds have been synthesised as possible lipophilic chemotherapeutics of the sulphonamide and sulphone series. Among

such compounds are the hydnocapric acid derivatives of N^4 -*n*-fatty-acyl sulphanilamides, $N^4:N^1$ -di-hydnocarpyl sulphanilamide, normal fatty-acyl derivatives of 4-nitro-4'-amino and 4:4'-diamino diphenyl sulphones, 4:4'-*bis*-adipylamido and 4:4'-*bis*-sebacylamido diphenyl sulphones, and the N^4 -*n*-caproyl derivatives of 2-sulphanilamido pyrimidine, 2-sulphanilamido-4-methyl and 2-sulphanilamido-4:6-dimethyl pyrimidines.

XIV. Synthesis of Phthalyl and ortho-Toluoyl Derivatives of the Sulphanilamides and Sulphones as possible Mycobacterial Antagonists

(DELIWALA, RAJAGOPALAN AND GANAPATHI)

In order to fit the sulphonamides and sulphones out better for use in the chemotherapy of tuberculosis and leprosy, synthesis has been effected of a series of *o*-phthalic and *o*-toluic acid derivatives of well known sulphonamides and substituted diphenyl sulphones as possible mycobacterial antagonists.

Phthalic and *o*-toluic acid derivatives were chosen for synthesis in view of the recent theory advanced by Shemiakin that the anti-haemorrhagic activity of substances of a vitamin K nature, intimately connected with the metabolism of mycobacteria, is due to phthalic acid which arises from the antihaemorrhagic substances. The study of the compounds designed as possible mycobacterial antagonists is expected to shed additional light on the mode of action of anti-haemorrhagic compounds and the relation between antihaemorrhagic action and antimycobacterial efficacy.

The twenty-six compounds synthesised include *o*-phthalic acid derivatives of sulphanilamidopyrimidine, sulphanilamido-4-methyl and sulphanilamido-4:6-dimethyl pyrimidines and of 4-nitro-4-acetamido-diphenyl sulphones and 4:4'-diaminodiphenyl sulphones. They also include N^4 -*o*-toluoyl derivatives of sulphanilamide, sulphathiazole, sulphathiazoline, sulphapyridine, sulphadiazine, sulphamerazine, and 5-bromo sulphadiazine and 5-bromo sulphamerazine. Other toluic acid derivatives prepared are $N^4:N^1$ -di-*o*-toluoyl sulphanilamide, 4:4'-*o*-toluoylamido-diphenyl sulphone, and 4-nitro-, and 4-amino-4'-*o*-toluoylamido diphenyl sulphones. In addition N^1 -*o*-toluoyl derivatives have been obtained from N^4 -formyl-, N^4 -acetyl-, N^4 -*n*-butyryl-, N^4 -valeryl-, N^4 -caproyl-, N^4 -*n*-heptoyl-, N^4 -cyclohexoyl- and N^4 -cinnamoyl-sulphanilamides.

XV. Preparation of 5:5'-Dichlorosalicil and Heterocyclic Derivatives from Dichlorosalicil and Related alfa-Diketones

(DELIWALA, RAJAGOPALAN AND GANAPATHI)

The highly favourable first reports about the antibacterial merits of 5:5'-dihalogenated salicils and the essentially sulphonamide-like nature of their action on bacteria, led to an improved method of pre-

paration of 5:5'-dichlorosalicil or "chlorosalicil" for ascertaining its usefulness in human tuberculosis.

The ineffectiveness of chlorosalicil on *P. pestis in vivo* directed attention to the synthesis of a series of biologically interesting heterocyclic ring-systems, particularly the imidazole and pyrazine types, starting with chlorosalicil, benzil, 2: 2'-dichlorobenzil, anisil, diacetyl and *ortho*-quinones, regarded as cyclic alpha-diketones.

A total of twenty-eight heterocyclic compounds have been synthesised in this connection. Synthesis of these furnished interesting information regarding the chemistry of imidazoles.

A few representative heterocyclics, now prepared, have been subjected to examination for efficacy *in vivo* against *P. pestis*, which definitely demonstrated reduction of the original toxicity for infected mice of the initial alpha-diketones following heterocyclic synthesis from the ketones. However these failed to exhibit any curative action. The failure in experimental plague of the compounds studied has not yet been correlated with their possible anti-mycobacterial activities.

XVI. Thiazole Derivatives

(K. D. KULKARNI)

2-Hydroxy thiazole has been prepared for the first time in good yields by the interaction of chlor-acetaldehyde with ammonium thiocarbamate. The method has been well standardised and excellent yields of 2-hydroxy thiazole have been obtained. In addition, several other derivatives of thiazole *viz.* 2-hydroxy-5-bromo-, 2-methyl-5-bromo-, 2-methyl-5-nitro-, 2-methyl-5-acetamino-, thiazoles have been prepared. The bromo-thiazoles have been successfully condensed with sodio-sulphinates to obtain corresponding sulphones and have been condensed with Na-salts of various sulphonamides to obtain sulphanilamidothiazoles, substituted in the thiazole ring.

XVII. Metabolism of *Penicillium Chrysogenum*

(V. SADASIVAN)

It has been found that there is an active phosphatase system in the mycelium of the mold which increases in activity with increase in age of culture. The enzyme has a pH optimum of about 8.7. No acid phosphatase could be detected. Cyanide inactivates the enzyme almost completely but not iodoacetate, fluoride cysteine or ascorbic acid. Metallic ions like Mg, Mn, Fe, and Co, do not activate it. The inhibition by cyanide is totally reversed by addition of zinc salts in concentra-

tions between M/500 and M/250. Concentrations of zinc above M/250 were found to be progressively toxic to the enzyme. This is in conformity with the observation that for penicillin production, zinc is essential but that there is an optimum concentration beyond which it is toxic to the fungus.

In the metabolism of the mold, there is a shift of pH to the acid side during the first phase of its growth, the reaction thereafter tending to become more and more alkaline. It is during this second phase that penicillin is present in the metabolism fluid in the highest concentration. This also appears to be the phase in which the phosphatase activity is the highest. Thus it leads to the conclusion that penicillin is probably present in the cells of the mycelium as a phosphoric acid ester and is released into the metabolism fluid when the enzyme activity increases and splits this ester. Further work is in progress.

XVIII. Typhoid Fever in the City of Bombay

(SOMAN)

Free diagnostic service in cases of typhoid fever is in continuous operation since 1938. As the incidence of typhoid fever in Bombay rose again during the years 1946 and 1947, Government sanctioned by the end of 1947, a typhoid unit scheme with additional staff and a motor car with which the Institute doctor collected blood from patients' homes. The scheme started actually functioning from middle of June 1948 and as a routine, blood was collected for triple widal test, clot culture, blood culture, total and differential counts and for malarial parasites. The number of samples tested serologically definitely increased due to presence of typhus infection in Bombay and 179 cases were diagnosed serologically during these years. As both these infections could not be clinically distinguished without difficulty Widal and Weil-Felix reactions were done on every sample as a routine. No case of para-typhoid B and para-typhoid C infection was diagnosed either serologically or culturally, but *Salmonella typhi murium* was isolated from a clot culture from a fatal case of continued pyrexia from Cama Hospital in 1948. The following table illustrates the results for the years 1947-1948. Figures for 1948 also include samples collected by the Institute doctor for the results obtained during last six months :—

Year	Total samples	Hospital samples	Private samples	Widal+		Widal— Clot—	Clot-cultures positive	
				Clot—	Clot+		B.ty.	B.para A
1947	1,575	1,189	466	98	114	261	185	27
1948	2,191	1,184	1,007	192	218	448	366	44

Total number of calls registered was 462 of which 38 were such, wherein the patient's address was incomplete or the patient refused to give blood. Thus 424 blood samples were collected and examined, of which 223 were diagnosed as cases of enteric, of which 132 were bacteriologically positive. Out of 132 cultures, 20 were *B. paratyphosus A*. As a routine, both blood culture (Hartley's broth) and clot culture (Glycerine-Peptide-Bile) were done on the same blood sample equally divided and hence the comparative positive results obtained by clot and blood cultures and the relative early appearance of growth in either of them was the interesting feature to be observed. Out of 132 bacteriologically proved cases, both blood and clot cultures were positive in 77, only blood in 20 and only clot in 35. From the following table, it can be seen that the clot culture method showed many higher positive results and they were obtained earlier than those obtained from whole blood culture.

	Positive in 24 hours	Positive in 48 hours	Positive in 5 days
Blood culture	.. 30 per cent.	55 per cent.	15 per cent.
Clot culture	.. 69 per cent.	23 per cent.	8 per cent.

Distribution of cases.—There were 402 calls from the city and 22 from the suburbs and this distribution according to wards when compared with that in previous years 1938 and 1939, showed that the same wards were heavily attacked.

Ward	.. A	B	C	D	E	F	G	Suburbs
Cases	.. 8	1	61	156	23	115	37	22

Examination of stools and urine.—In spite of reporting serologically or bacteriologically positive cases, no samples of stools or urine, were received from the private practitioners to see whether the patients were free from excreting these organisms and that they did not act as carriers. D. E. C. medium has been found to be very satisfactory for such investigation when used alone or in combination with bismuth sulphite agar.

XIX. Cholera in Bombay City

(SOMAN)

Since 1943, the strains of *V. cholerae* isolated from stools of cholera cases in Bombay have been serologically studied and their sub-types recorded. The serological types are known to change from year to year and from epidemic to epidemic but the conditions under which this change is brought about are not exactly known. From the very beginning of the year 1947, the Arthur Road Hospital had started sending stools or rectal

swabs of cases of vomiting and diarrhoea. The first case, however, from which cholera vibrio was isolated, occurred in the month of May. This was a local case, giving history of having gone to Nasik and having eaten eatables brought from there. In the month of June, however, no samples were received for examination for cholera. But July and August showed a sudden flare up with 345 and 734 samples received and 123 and 102 positive cholera isolations by culture. Since September, it showed a steady decline. The number of cases from which vibrio cholera was isolated being 49 in September, 10 in October, none in November and the last case to be recorded was on 1st December. Samples continued coming from Arthur Road Hospital throughout the year 1948. The total number of samples examined from May 1947 to November 1947 was 1,657 and 314 strains of *V. cholerae* were isolated and 46 strains during the year 1948. Six strains of *V. cholerae* were isolated from stools received from Ahmedabad during 1948 and they all belonged to Ogawa sub-type.

The serological types and the total number isolated in the years 1943, 1945, 1947 and 1948 are shown in the table below ;—

Year				Total	Ogawa	Inaba
1943	..	..	..	34	32	2
1945	..	..	..	166	165	1
1947	..	..	..	314	9	305
1948	..	..	..	46	46	..

No cholera organisms were isolated in the intervening years 1942, 1944 and 1946.

Samples of stools were also sometimes sent along with the rectal swabs. Not all stools were rice-watery. Of 150 that were received, only 62 were rice-watery which gave 75% positive cholera isolation; 13 contained blood either alone or along with mucus, presented an appearance of typical dysenteric stools, 5 of which were positive for cholera (38%). The rest 58 were diarrhoea stools and showed 17% to be positive by culture.

During 1948, the Director of Public Health and the Director, Haffkine Institute collaborated in a joint field trial of a drug Sodium Phthalyl-Sulphacetamide in the treatment of cholera cases at Erandol for 2 months and a small unit was sent from this Institute for bacteriological diagnosis and other laboratory investigation. Over 200 samples

of stools were examined and 40 cases were diagnosed as true cholera and 34 strains were isolated; majority of which belonged to the serological sub-type Ogawa.

XX. Typhus in Bombay City

(SOMAN)

During the period under review, a further supply of specific rickettsial antigens was available through the courtesy of American workers at the National Institute of Health, Bethesda, Lederle Laboratories, New York and Rocky Mountain Laboratories, Montana. Sera from typhus cases and from wild and domestic rats from Bombay were tested with these antigens by Complement fixation method. Attempts were also made to isolate human, rat and mite strains by suitable animal inoculation tests.

Serological diagnosis.—Sera from 3,897 febrile cases were examined by Weil-Felix test and 268 samples from 179 cases were found to be positive for presence of typhus infection. The history records of these cases showed that the cases were mild, without rash and delirium and clinically indistinguishable from mild enteric infection except by laboratory tests.

(a) *Study of typhus sera by complement fixation and Rickettsial agglutination tests.*—The importance of complement fixation test in the epidemiological studies of typhus was duly stressed in a previous report, on account of the failure of Weil-Felix test to distinguish the different types in the OX 19 group. For performance of complement fixation test, Bengston's technique was adopted and 1 in 5 titre of the serum was taken as diagnostic. 90 Wasserman sera when tested in this way, were all negative. 238 samples derived from 161 cases were tested by this method and 94 of these were diagnosed as sera from murine typhus, 3 as from epidemic louse typhus and 3 provisionally as from tick typhus. In 2 cases, the sera fixed the R. M. S. antigen in a titre of 1 in 5 and 1 in 10 and in 2 other cases, sera fixed the epidemic antigen. Sera from epidemic typhus cases were received from Gharwal district, in which louse-borne or epidemic type was reported. Usually the results of Weil-Felix and complement fixation tests compared favourably but few cases with high OX19 titres were encountered, in which these tests were negative.

(b) *Rickettsial agglutination test.*—The rickettsial agglutination test was carried out using murine and epidemic rickettsial suspensions obtained through the courtesy of Dr. C. E. van Rooyen of Connaught Laboratories, Toronto. 1 in 100 titre of the serum was accepted as diagnostic of typhus infection. On the whole, the results of complement fixation and rickettsial agglutination ran parallel, because majority of samples tested were collected after the 10th day of disease. Some of our sera were checked by the workers in the National Institute of Health, Bethesda and gave comparable results,

The table below is representative of the general results.

No.	W. F.		R. A.		C. F.		Nature of typhus
	X19.	X2.	M.	E.	M.	E.	
1	400	—	100	100	10	—	Murine type
	800	—	400	400	40	—	
2	250	—	50	800	5	40	Louse or epidemic type
3	250	—	200	400	20	40	
4	400	—	—	—	—	—	Do. Tick-borne RMS type.
5	100	—	—	—	—	—	
						5	Do.

W. F.=Weil-Felix Test

M.=Murine antigen

R. A.=Rickettsial Agglutination

E.=Epidemic antigen

C. F.=Complement fixation

RMS=Rocky Mountain Spotted Fever Antigen

Isolation of strains from typhus cases.—Patients' blood was inoculated intraperitoneally into male guinea-pigs or in their absence in laboratory-bred white rats. Sixty-one attempts were made. Out of 8 guinea-pigs so inoculated 2 showed febrile reaction, only 1 showed Neill-Mooser reaction in addition and the rest did not react at all. Sera of these positive and negative reactors was tested for complement fixing antibodies. In one negative reactor, the antibodies were clearly demonstrated in its serum, thus suggesting the possibility of silent infection in such animals.

Complement fixation test with sera of wild and domestic Rats.—As previously reported, 3 species of Bombay rats were taken for study; *Rattus rattus*, *Rattus norvegicus* and *Gunomys kok*. A fourth species, *Suncus caeruleus*, has been added this year. 355 rat sera were examined during the period under review and as expected, *Rattus norvegicus* showed the highest percentage of positive infection for murine typhus. Last two years' findings regarding the murine typhus infection amongst the different rat population of Bombay City are summarized below:—

Table showing the results of C. F. test on rat sera for two consecutive years

Species examined	Total No. examined	Positive C. F. Test	Percentage Positive
<i>R. Rattus</i>	323	62	19.2
<i>R. Norvegicus</i>	193	84	43.5
<i>Gunomys kok</i>	208	13	6.2
<i>Suncus caeruleus</i>	15	0	0.0
	739	159	21.5

Isolation of murine strains from Bombay rats.—One more strain of murine virus was isolated from a pool of three *R. norvegicus* brains, passaged into a male guinea-pig. All the rats showed C. F. antibodies in their sera. The guinea-pig reacted typically with fever and scrotal swelling in the acute phase and C. F. antibodies during convalescence. During the year 1948, a new species of rats *Suncus caeruleus* was available for examination in small numbers. Their sera were tested by complement fixation test. As shown in the table, all the sera of this species were negative by this test. 8 brain pooled suspensions from 23 rats of this species were inoculated in the guinea-pigs. Four guinea-pigs showed regular febrile reaction, but no strain has yet been isolated. Further work regarding this is in progress.

Survival of murine virus in laboratory-bred white rats.—This experiment was planned last year to throw some light on the epidemiology of typhus infection in Bombay. Two laboratory-bred white rats were inoculated with two different human strains of murine virus and the third one was inoculated with a rat strain of the same virus, and kept under observation. Brain of each rat was passaged separately into three male guinea-pigs at the end of 472, 510 and 522 days respectively. All the three guinea pigs showed febrile reaction. Neil-mooser reaction was observed in one and the complement fixation test was positive in two. This experimental observation, though solitary one, clearly establishes the fact that murine virus is capable of surviving for a long period of over a year in white rats and this very probably holds good for wild rats too. Pollard and Auguston (1945) in U.S.A. have also reported that the murine typhus agent could be isolated from the brain of rat as long as 370 days after inoculation. Thus the wild rats once infected in nature, maintain such infection for a very long time and act as reservoirs.

Mite-typhus.—Although sera from febrile cases were examined all the year round for presence of XK typhus, it was only after monsoon a few cases were detected serologically. 30 samples derived from 19 cases showed titres above diagnostic level. The number of such cases being very small, mice inoculation was done in all cases and 5 strains could be isolated.

Trombiculid larvae were collected from different species of rats, the total number being 2,628. 1,558 were picked up from *Gunomys kok*, 990 from *Rattus norvegicus* and only 50 from *Rattus rattus*. The small number of *Suncus caeruleus* examined, showed no mite infestation. 3 species, *Tr. indica* and *Tr. paltai* were identified and *Asco-schongastca hirst* was also identified. One more lot has been sent to the National Institute of Health, Bethesda, U.S.A. and the report is awaited. Attempts were also made to isolate *R. orientalis* strains from mites. Five different pools of larval mites varying from 40 to 80 in each pool were inoculated into white mice, but no strain has been so far isolated. In view of the small number of XK typhus cases and with peculiar short-term seasonal incidence, it is difficult to locate the arthropod vector

unless the work is carried on from year to year. The following table illustrates the number of cases of murine and mite typhus during 4 years.

	1945	1946	1947	1948
Total number of samples examined	1,566	1,561	1,649	2,228
Murine typhus	102	78	68	93
Mite typhus	10	15	11	7

XXI. Quantitative Determination of p-p'Dichloro-Diphenyl-Trichloroethane in Commercial DDT Dusts and Powders

(DESHPANDE, BANERJEE AND TAMHANE)

The dusts marketed by the commercial firms usually contain technical DDT diluted with Kaolin. The insecticidal properties of DDT depend mainly on the amount of the p-p'isomer of dichloro-diphenyl-trichloroethane. The method used for the dehydrohalogenation of DDT with normal alcoholic Potassium Hydroxide was studied at varying concentrations, time and temperature. The studies have shown that the amount of the p-p'isomer can be best determined by using Normal alcoholic KOH and carrying out the reaction at a temperature of 20°C for a period of 30 minutes. The method developed gives comparable results with the method official in the Indian Pharmacopoeial List 1946 which is very time-consuming.

XXII. The Effect of restricted intake of Protein (Casein) in an otherwise adequate Diet on the Histological and Biochemical Changes in the Liver of Rats

(RADHAKRISHNA RAO, DATTA AND KRISNAN)

In our previous experiments (reported in the last annual report), rats fed on diets low in protein (casein) and deficient in vitamins of the B₂ complex did not show massive necrosis. This is in contrast to the findings of Himsworth and Glynn (1944) who claimed to have produced massive hepatic necrosis in rats by feeding low protein diets in which the daily intake of protein (casein) was between 200 and 500 mg. It was, therefore, thought worthwhile to study the histological and biochemical changes in the liver of rats on a restricted intake of casein at different levels in an otherwise adequate diet. The average results are given below :

Daily Casein intake	Initial weight in g.	Final weight in g.	Daily Calories intake	Liver weight g./100g body wt.	Liver water g./100g body wt.	Liver Fat g./100g body wt.	Liver protein g./100g body wt.	Liver Ash g./100g body wt.
(a) 1.2g.	37.8	170.3	32.66	3.24	2.28	0.17	0.65	0.05
(b) 0.6g.	37.5	149.5	28.6	2.87	2.04	0.17	0.55	0.04
(c) 0.4g.	36.0	139.0	26.7	3.16	2.19	0.31	0.51	0.04
(d) 0.2g.	38.8	89.0	19.3	4.16	2.81	0.48	0.65	0.05

The results show that the growth and caloric intake of the different groups of rats parallel the daily casein intake. With the lowering of the daily casein intake, there is a definite tendency for the liver weight to increase and this increase is chiefly due to the increase of water and fat content.

The average values of the total plasma protein concentration and choline content of the liver in mg/g of fresh liver tissue in different groups of rats are given below :—

Protein (Casein) intake ..	1.2g.	0.6g.	0.4g.	0.2g.
Total protein g/100 c-c. Plasma ..	6.83g.	6.11g.	5.52g.	4.81g.
Choline mg/g of fresh liver ..	2.14 mg.	1.89 mg.	1.43 mg.	1.55 mg.

The plasma protein concentration is consistently lowered as the daily intake of casein was reduced. The choline content of the liver also shows a similar decrease.

On microscopical examination, the liver showed no pathological changes except slight pallor or mottling in the low protein groups.

Histology.—On microscopical examination, the liver in animals which received 1.2 g. and 0.6 g. of casein per rat per day showed no pathological changes. Animals which received 0.4 g. and 0.2 g. of casein per rat per day, on the other hand, showed slight fatty change of the hepatic parenchyma around the central veins. But, in marked contrast to the animals in the low protein groups which were deprived of vitamins of the B₂ complex, none of the animals in this group showed marked fatty change in the hepatic parenchyma around the central veins, accumulation of ceroid pigment or diffuse hepatic fibrosis.

XXIII. Experimental production of Hepatic Cirrhosis in Rats by High Fat-Low Protein and High Carbohydrate-Low Protein Diets and the Effect of Lipotropic Factors like Choline and Methionine on the Hepatic Lesions

(RADHAKRISHNA RAO, DATTA AND KRISHNAN)

Twenty four rats were fed on a high fat diet consisting of 50 per cent. hydrogenated vegetable fat, 6 per cent casein, 40 per cent. carbohydrate (starch and sucrose), 3 per cent. salt mixture and 1 per cent. cod-liver oil. Daily supplements of thiamin hydrochloride, riboflavin, pyridoxine and calcium pantothenate were given to the animals.

The high carbohydrate diet consisted of 85 per cent. carbohydrate (starch and sucrose in equal parts), 6 per cent. casein, 5 per cent. fat, 1 per cent. cod-liver oil and 3 per cent. salt mixture. The vitamin supplements were the same as on high fat diet. Twelve rats were given this high carbohydrate diet.

The action of choline in doses of 4 mg. per rat per day and methionine in doses of 20 mg. per rat per day was studied in two separate groups of rats (each group consisting of 12 rats) which were fed on the high fat diet.

A considerable number of rats receiving high fat diet developed oedema and ascites. A few of them died while others were killed during periods ranging from 33-110 days. The livers were enlarged in almost all cases and contained on an average 25.4 per cent. fat on fresh liver basis. The total plasma protein was 3.77 g/100 c.c. plasma which is much below normal.

On microscopical examination of sections of the liver, no massive necrosis or post necrotic scarring was evident, but fatty change was present in varying degrees and a number of them showed diffuse hepatic fibrosis; in some, typical cirrhosis with ceroid pigmentation was found.

On the high carbohydrate diet, the animals lived much longer than on the high fat diet. Animals in this group showed varying degree of enlargement of the liver, which appeared fatty and granular in many cases. The consistency of the organ also varied from animal to animal.

Histologically, marked fatty infiltration in the liver was found in almost all the animals. About half the number killed after 150 days showed replacement fibrosis, particularly around the central veins. Typical cirrhosis with ceroid pigmentation was found in one instance.

Action of choline.—Choline in doses of 4 mg. per rat per day given along with the high fat diet had no effect on the daily food consumption or on the survival period of the rats. The fat content of the liver, however, was considerably reduced and was only 9 per cent. as compared to 25.4 per cent. in untreated cases.

Microscopical examination of sections of the liver corroborated the chemical findings. The fatty change in the liver was much less marked in this group of animals as compared to the control animals. Similarly, fibrosis was also less marked and there was no accumulation of ceroid pigment. From the histological examination, however, it appeared that a daily supplement of 4 mg. choline per rat is not sufficient to completely prevent the fatty change in the liver.

Action of methionine.—Animals receiving 20 mg. of methionine per rat per day along with the high fat diet lived much longer than those which received choline. There was no oedema or ascites in this group of animals. Animals were killed during periods varying from 99 to 162 days.

The liver was slightly pale in colour but otherwise was normal. There was moderate fatty change in the liver with slight accumulation of ceroid pigment. As compared to the choline group, the fatty change in the liver was more marked in this group of animals. Slight fibrosis around the central veins was also observed in a few animals.

XXIV. Treatment of Decompensated Portal Cirrhosis with High Caloric, High protein Diets with Vitamin Supplements

(RADHAKRISHNA RAO, DATTA AND GOVIND)

This investigation was carried out in collaboration with Dr. N. J. Modi, M.B.B.S., M.R.C.P. (Lond.), in the medical unit of the Goculdas Tejpal Hospital, Bombay.

Twenty patients were treated with high protein diets which included skimmed milk powder, egg protein, fresh milk and casein hydrolysates. Vitamin supplements were given in the form of yeast tablets and injections of vitamin B₂-complex and liver extract in some cases. The majority of the patients came from the poorer classes and their dietary histories revealed gross deficiency in calories, protein and vitamin intake. Ten out of the 20 cases gave history of having had attacks of malaria. Nine cases were addicted to alcohol. Past history of dysentery was present in four cases and jaundice in only two cases.

The presenting symptom of all patients was enlargement of the abdomen, varying in duration from 15 days to two years. All the cases showed evidence of fluid in the abdomen. Spleen was enlarged in 8 cases and liver was found enlarged in four cases only. The level of serum proteins was estimated before, during and after treatment, as this afforded an objective evidence of the course of the disease. Total protein, albumin, globulin and Euglobulin content of blood serum was estimated.

A common observation in all the patients before treatment was the fall in albumin and rise in globulin fraction of the serum proteins, thus showing an altered albumin/globulin ratio from the normal. In the globulin fraction, the Euglobulin showed marked increase.

The period of observation during treatment was on an average 5 to 6 months. The results of therapy at the hospital have been quite encouraging. Patients treated with high protein diet showed definite clinical improvement. Ascites, oedema and other manifestations of the disease disappeared in many instances. Clinical improvement was attended by a change in the serum protein level towards normal values.

XXV. On the Nutritive value of Vanaspati

(RADHAKRISHNA RAO AND DESHPANDE)

(A) *Animal Experiment*.—36 young albino rats, 28 days old were divided into three groups, each group consisting of 6 males and 6 females. The rats were fed on "Poor Madras Diet", with the following three different fats at a 5% level :—

- (1) Crude Ground Nut Oil
- (2) Vanaspati M. P. 37°C
- (3) Pure Cow's ghee

The feeding was continued for three months after which period the rats from the same group were mated together. When the females were pregnant, they were removed to individual cages.

A record of weights of individual rats was maintained during the first three months of the feeding. It was found that there was no significant difference in the average weights in the three different groups of rats at the end of three months.

The ability of the females in the three different groups for reproduction and lactation was also recorded. Thus it was found that there was one sterile female in each of the three different groups of rats. The number of young ones varied in the different groups but here again there was no significant difference. In all the groups the young ones were emaciated and subnormal in weight.

The animals of the 2nd generation were weaned after about two months and their growth on the respective diets observed for further three months. In all the three groups the growth was stunted and the animals could not be mated for third generation.

(B) *Human Feeding Experiment.*—This was conducted at the David Sassoon Industrial School, Matunga, Bombay, where there were about 400 boys ranging from 8 to 15 years in age. 185 boys between the ages of 11 and 14 years were selected for the experiment. The boys were divided into four batches with respect to their heights, weights and clinical conditions. The batches were fed for one year on the usual institutional diet with one oz. of oil or Vanaspati.

A record of their weights and heights was maintained as also that of the nutritional state.

On the whole it was found at the termination of the experiment that there is no statistically significant difference in the average increase in heights and weights and also in the nutritional state of the boys getting oil and Vanaspati. No deleterious effects of feeding Vanaspati were observed.

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