



Report of the Haffkine Institute for the Year 1949

BY

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BOMBAY

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1949

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1949

PART I

GENERAL

I. Staff

DIRECTOR

Major-Gen. 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 29th April 1949 to 20th June 1949 and from 17th September 1949 to 29th October 1949. Officiating as Director from 15th December 1949.

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

Shri. M. V. Shirsat, M.Sc., Officiated as Assistant Director, I/c Department of Chemotherapy from 17th September 1949 to 16th December 1949.

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.

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

Shri B. V. Patel, B.Sc. (Bom.), B.Pharm. (Lond.), Ph.C. (Gr.Br.), M.P.S., Officiating Assistant Director, I/c Department of Pharmacology upto 30th June 1949.

Shri K. G. Ananthanarayanan, M.Sc., Officiated as Assistant Director, I/c Department of Pharmacology from 1st July 1949 to 16th November 1949.

II General Review

During the year under review the Institute continued to be of as great a service to the public as in the years previous to it. The fact that the demand for biological products made available by it has been steadily on the increase, should be taken as the measure of the confidence the public has learnt to place on their efficiency and the guarantee of their purity. That their rates are considerably below the cost of similar products in the market must also be accounted for as another factor that has contributed to the increased demand. The sales amounted to Rupees Twenty-four lakhs while the expenditure was Eighteen lakhs during the financial year 1948-49.

Nine million millilitres of plague vaccine and six million millilitres of cholera vaccine were issued during the year under report. The Department of Vaccines has been now organised to produce, on an average, 25 million millilitres of vaccines a year, but the organisation is elastic enough to enable us to step up the production to 50 million millilitres in times of emergencies. The demands for vaccines came from all over India.

Research work with the curative agents for human plague infection has continued. Both experimental and field work has shown that Streptomycin exerts a very powerful curative action in bubonic plague and there are indications that it would exert similar action in pneumonic plague. In experimental plague infection, both Aureomycin and Chloromycetin showed equally powerful curative action although in the case of the latter drug a rather larger dose was required to be given to produce the result. If Aureomycin, which is given orally, would give the same results in human plague infection in the field, it would greatly facilitate the question of plague treatment in villages where the plague infection primarily occurs.

The demands for the use of Antitoxins and Sera continued to be on the increase and came from all over India. The Department being primarily organised to meet the needs of the hospitals of Bombay State and the capacity for production being limited, many extra-provincial buyers could not be supplied with their requirements. The only solution to the problem appears to be an expansion of the Department to meet the increased demands. The construction and equipment of the new low-temperature laboratory is not yet completed, but when this is done, the alcohol precipitation methods of serum proteins for which Dr. Lahiri was deputed abroad will be tried and if these prove successful it will enable the Department to issue purer and more stable antitoxins and sera.

The Department of Chemotherapy has now undertaken the regular manufacture of Sulphathiazole, and during the year, about 2,000 lbs. of Sulphathiazole and 3,500 ampoules of injectible Sodium Sulphathiazole solutions were manufactured. Many improvements have been carried

out which would result in reduction of costs and simplification of working methods—experiments have been under way and are almost nearing completion to take up the manufacture of Sulphamerazine on a pilot plant scale and it is hoped to take up the routine manufacture of Sulphamerazine during the current year. Recently, the Department has undertaken the preparation of Hydnocreol Oil injectible and “Sulphetrone injectibles” for supplying to the hospitals and clinics in the State for the treatment of Leprosy patients. Research work is in progress on the Chemotherapy of Leprosy, tuberculosis and malaria.

The Institute has been fortunate in securing the services of an able Pharmacologist, Dr. N. K. Dutta, to take charge of the Department of Pharmacology. Tests carried out in this Department in pursuance of the Drugs Act, 1940, have shown that while amongst the 95 imported medical preparations tested in the laboratory about 92 per cent were found to be of standard quality, amongst the 300 odd samples of drugs and chemicals tested under the Drugs Act, 1940, about 50 per cent proved to be either below standard or did not conform to the claim made by the manufacturers. There is thus no doubt that adulteration is practised to a great extent in this trade and stringent control is necessary if the public is to be protected from this spurious racket.

The Department of Nutrition rendered useful service to Government on all nutritional questions and carried out hundreds of tests on food-stuffs with a view to find out their suitability for human consumption. The Department also served as a centre for training in practical nutrition work. A short-term human feeding experiment with groundnut cake atta was conducted by the Department and it was found that the groundnut cake atta can be used to the extent of 10 per cent and not exceeding half an ounce per day in ordinary preparations without any digestive or other disturbances. The Department of Nutrition and Experimental Pathology at this Institute is now amalgamated with the Department of Nutrition of the Government of Bombay. The new Department of Nutrition is located for the present at this Institute under the administrative control of the Surgeon General and Dr. M. V. Radhakrishna Rao, Assistant Director, Haffkine Institute, has been put in charge of this Department.

In spite of the opening of a treatment centre at the Municipal K. E. M. Hospital at Parel, the number of patients attending the Out-Patients Department of the Department of Virus Diseases did not diminish. The demand for rabies vaccine from the different antirabic centres in the State remained high as in the previous year. The Department continued to serve as an inoculation centre for Yellow Fever and other vaccines for international travellers.

Demands for the intravenous fluids like Glucose Saline and Casein

The Department of Entomology has been without an Entomologist since Dr. Sharif left the Institute in November 1948. Entomologists as well as Pharmacologists appear to be rare. A plague training centre is to be established in the Institute when the World Health Organisation team arrives in the country to assist the public health authorities in the country in the eradication of plague infection from endemic centres. A competent Entomologist will then be required and every effort is being made to find one of requisite standing and experience to take charge of the Department and carry out the training scheme proposed by the W. H. O. team.

The Department of Clinical Pathology gave diagnostic aid to various Government and Municipal Hospitals in the City and free diagnostic aid was given to private medical practitioners in case of infectious disease. As a result of this, the work of the Department has increased considerably and over 20,000 samples were examined during the year. The Blood Bank Department is doing useful work in the supply of dried plasma to hospitals and practitioners and the demand for this is on the increase. The response from the voluntary donors, however, continues to be as unsatisfactory as before, and increased demand for the plasma could only be met because of a better response for blood donations from the prisoners in jails.

III Department of Vaccines

During the year under review, the demand for the various vaccines was lower than that of the previous two years. While 40 million millilitres of vaccines were issued in 1947 and 23 million millilitres in 1948, during the present year the demand was for 15 million millilitres only. This was due primarily to lesser incidence of plague and cholera during this year than during the previous two years. The demands were received from different parts of India.

A. PLAGUE VACCINE

During the year under report the quantities of plague vaccine issued monthly are detailed below:—

Months	Quantity in millilitres					
January	..	..	..	..	..	918,214
February	..	..	..	..	..	1,086,073
March	..	..	..	..	..	358,721
April	..	..	..	..	..	463,883
May	..	..	..	..	..	100,832
June	..	..	..	..	..	40,650
July	..	..	..	..	..	109,448
August	..	..	..	..	..	460,640
September	..	..	..	..	..	1,360,499
October	..	..	..	..	..	1,866,220
November	..	..	..	..	..	1,935,580
December	..	..	..	..	..	390,739
						9,091,499

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
1947-1949 (three years)	48,719,471

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

	Quantities in millilitres
INDIA	1949
Bombay Province	2,359,513
Madras Province	500,850
Bengal, Bihar and Assam	501,550
United Province of Agra and Oudh	2,092,750
Punjab	201,920
North West Frontier Province	45,100
C. P. and Berar	277,845
Delhi Province	31,103
Central India	14,378
Total	6,025,009
INDIAN STATES	1949
Hyderabad Deccan	2,252,800
Mysore	2,570
Patiala	80,000
Gwalior	13,180
Benares	26,600
Panna	6,000
Chhatarpur	45,900
Rewa	53,500
Total	2,480,550
FOREIGN COUNTRIES	

B. CHOLERA VACCINE

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

Months						Quantity in millilitres
January	..	..	..	..	..	25,390
February	..	..	..	..	..	93,901
March	..	..	..	..	..	105,715
April	..	..	..	..	..	219,273
May	..	..	..	..	..	698,457
June	..	..	..	..	..	944,362
July	..	..	..	..	..	814,508
August	..	..	..	..	..	651,478
September	..	..	..	..	..	167,685
October	..	..	..	..	..	856,729
November	..	..	..	..	..	761,970
December	..	..	..	..	..	832,032
Total ..						6,171,500

C. T. A. B. VACCINE.

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

Months.						Quantity in millilitres
January	..	..	..	..	..	10,730
February	..	..	..	..	..	31,144
March	..	..	..	..	..	18,836
April	..	..	..	..	..	18,575
May	..	..	..	..	..	14,930
June	..	..	..	..	..	58,272
July	..	..	..	..	..	59,313
August	..	..	..	..	..	81,957
September	..	..	..	..	..	90,215
October	..	..	..	..	..	69,063
November	..	..	..	..	..	31,458
December	..	..	..	..	..	30,958
Total ..						506,451

D. THE MEDIA SECTION.

This Section's work falls into three categories :—

1. The Production of media for the manufacture of vaccines.
2. The Production of media for routine work carried out at the Institute.
3. The manufacture of intravenous fluids.

1. Casein Hydrolysate medium as standardized at this Institute is being manufactured on a large scale for the production of plague and cholera vaccines. During this year, 18,339,000 ml. of plague medium and 8,073,000 ml. of cholera medium were prepared. For the preparation of T. A. B. Vaccine 117,200 ml. of Agar was prepared in Acid Digest Broth.

2. Different types of media were prepared for the routine work of the various departments of the Institute such as Clinical Pathology Department, Vaccine Department, etc. etc. For testing sterility of vaccines, toxins, anti-toxins and fluids used for intravenous administration, the aerobic and anaerobic media were prepared on a large scale. During the year, 2,450,000 ml. of aerobic medium and 2,450,000 ml. of anaerobic medium were prepared.

3. The manufacture of intravenous fluids was started in the year 1946. Since then there has been an increasing demand for these fluids. The following table shows the number of bottles manufactured during 1948 and 1949 :—

Name of the Fluid	Number of bottles manufactured during 1948	Number of bottles manufactured during 1949
Glucose Saline	10,604 (of 500 ml. each).	21,661 (of 500 ml. each).
5% Glucose in Dist. Water	141 (of 500 ml. each).	817 (of 500 ml. each).
Normal Saline	705 (of 500 ml. each).	847 (of 500 ml. each).
Hypertonic Saline	865 (of 500 ml. each).	727 (of 500 ml. each).
25% Glucose in Dist. Water	3,320 (of 100 ml. each).	4,543 (of 100 ml. each).
Protein Hydrolysate	1,100 (of 400 ml. each).	4,008 (of 400 ml. each).

All the intravenous fluids are utilised by the private as well as Government hospitals. Arrangements are made to supply our products all over the State of Bombay. Due to the comparatively low prices, and high quality of our products, they have gained wide popularity and consequently the demands are going up every year.

IV. Department of Anti-toxins and Sera

The year 1949 had to be devoted more to routine production work than to advancement of technique and scientific knowledge. In spite of repeated refusals to supply extra-provincial demands, request continued to pour in. It proved a very difficult task to meet some of the

efficiency. Newer scientific works had, thus, to suffer during this year. Nor could the rate of production be increased significantly. Now it seems to be certain that with the present set up no further increase in production is possible.

Epizootic Lymphangitis of horses still remained in existence and took a toll of 14 horses as against 11 during the last year (1948) and 61 during 1941. Every preventive measure is being taken to control the disease.

1. *Horses*.—One hundred and thirty nine horses were used during 1948, and 128 during 1949, for the production of various anti-toxins and sera. A number of preliminarily immunized horses varying between 20 to 30 were always kept in readiness 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 :

Antit-Toxins	Number of horses used	
	1948	1949
1. Tetanus Antitoxin	52	55
2. Diphtheria Antitoxin	37	33
3. Anti-snake-venom Serum	13	15
4. Welchii Antitoxin	20	14
5. Septique Antitoxin	5	1
6. Oedematiens Antitoxin	2	2
7. Anti-plague Serum	7	4
8. Anti-dysentery (Shiga) Serum	2	Nil.
9. Special Anti-allergic Serum	Nil.	3
10. Normal Horse Serum	1	1
Total ..	139	128

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 toxins of tetanus and diphtheria were specially reserved for the preparation of respective toxoids for human use.

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	
	1948	1949
1. Tetanus	293	223
2. Diphtheria	180	269
3. Welchii	76	52
4. Septique	26	<i>Nil</i>
5. Oedematiens	6	<i>Nil</i>
6. Shiga	9	<i>Nil</i>

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 801,130 ml. The quantities prepared during each year were as follows :

Types of Antitoxins or Sera	Quantities in millilitres	
	1948	1949
Concentrated and purified antitoxins ..	6,49,100	7,47,800
Natural Serum	1,19,960	57,330
	7,69,060	8,05,130
1. Tetanus Antitoxin	3,50,200	4,03,700
2. Diphtheria Antitoxin	1,16,990	1,33,500
3. Gas-gangrene Antitoxin	1,04,710	1,16,800
4. Anti-snake-venom serum	77,200	93,800
Total concentrated Sera ..	6,49,100	7,47,800
5. Anti-dysentery serum (natural) ..	22,670	<i>Nil.</i>
6. Anti-plague serum	71,500	16,120
7. Anti-allergic serum	<i>Nil.</i>	17,960
8. Normal horse serum	25,790	23,250

4. *Lyophilization of sera*.—During 1949 a total volume of 93,800 millilitres of polyvalent anti-snake venom serum was processed for preservation by drying from frozen state. After lyophilization, 58,270 ml. of serum was issued for use in man during 1949.

5. *Supply*.—The following quantities of antitoxins, sera and toxoids were issued during the years 1948-1949 :—

	Quantities supplied in millilitres	
	1948	1949
1. TETANUS ANTITOXIN		
(a) Prophylactic—3,000 I. U. in 2 c.c. ..	1,84,032	2,47,474
(b) Curative—20,000 I. U. in 10 c.c. ..	1,53,750	1,29,680
(c) Curative—10,000 I. U. in 5 c.c. ..	5,775	7,060
(d) Highly concentrated—20,000 I. U. in 4 c.c. ..	25,024	73,512
2. DIPHTHERIA ANTITOXIN		
(a) Prophylactic—1,000 I. U. in 2 c.c. ..	1,738	7,234
(b) Curative—10,000 I. U. in 10 c.c. ..	68,760	62,270
(c) Curative—5,000 I. U. in 5 c.c. ..	1,760	4,055
(d) Highly concentrated—10,000 I. U. in 5 c.c. ..	2,230	23,120
3. GAS GANGRENE ANTITOXIN		
(a) 3,000 I. U. Welchii antitoxin in 10 c.c. ..	51,820	79,840
(b) 9,000 I. U. Welchii, 4,500 I. U. Septique and 9,000 I. U. Oedematiens antitoxins in 15 c.c. ..	2,400	5,955
4. ANTI-PLAGUE SERUM IN 20 c.c.		
.. ..	39,380	3,500
5. LYOPHILISED POLYVALENT ANTI-SNAKE-VENOM SERUM IN 10 c.c.		
.. ..	54,500	58,270
6. ANTI-DYSENTERY SERUM		
(a) Natural, 4,000 I. U. in 20 c.c. ..	3,880	3,900
(b) Concentrated, 4,000 I. U. in 5 c.c. ..	690	2,200
7. TETANUS TOXOID		
(a) in 10 c.c. ..	9,630	670
(b) in 5 c.c. ..	760	915
(c) in 1 c.c. ..	673	318
8. DIPHTHERIA TOXOID ALUM PRECIPITATED		
(a) in 5 c.c. ..	65	820
(b) in 1 c.c. ..	319	188
9. LYOPHILISED SCHICKTOXIN Sufficient for 25 tests		
.. ..		1
10. NORMAL HORSE SERUM IN 10 c.c.		
.. ..	3,690	9,710

6. *Training*.—During the year two officers from the Drug Research Laboratory, Jammu and Kashmir, were deputed for training.

Shri Mir Habibullah, M.Sc., Chemist of the Kashmir Drug Research Laboratory, while under training at the Institute from 17th January 1949 to 28th February 1949, followed the working of this department for about a week or so.

The second officer, Dr. J. D. Kohli, L.T.M., L.S.M.F., Assistant Bacteriologist, while on deputation here from 23rd January 1949 to 6th May 1949 devoted a considerable period of his stay in this department following, observing and in some cases actually carrying out inoculation and titration experiments.

Dr. C. V. Natarajan, M.B.B.S., D.P.H., F.R.I.C., F.A.Sc., Director of Public Health Laboratory, Bangalore, was here from 29th August 1949 to 7th September 1949 and went round the departments following the workings of different sections, particularly the standardisation of the Antisera.

V. Department of Entomology

The work of the Department suffered considerably owing to the absence of an Entomologist after Dr. Sharif's departure. Internal arrangement was, however, done to carry out the routine work. Technical assistance was given by this Department to Health Authorities of the State and many enquiries about plague, fleas, other insects and rodents and their control were attended to.

Samples of rat-traps for expert advice were received from different manufacturing firms and opinion was given on them.

The 1949 class of Sanitary Inspectors, divided into two batches, was given a short course of demonstration and lectures in the subject of rat examination and destruction.

Two courses of training in Plague and Anti-plague measures were conducted for the benefit of Sanitary Inspectors and other Public Health personnel of the Province. The first course was held from 28th February 1949 to 12th March 1949 and 23 Sanitary Inspectors attended this course. The second course was held from 5th September 1949 to 20th September 1949, and was attended by one Medical Officer and 17 Sanitary Inspectors.

In addition, Dr. B. K. Mahajan, Plague Control Officer of the Plague Organisation under the Director of Public Health for the Government

A. EXAMINATION OF BOMBAY CITY RATS

A permanent organisation is maintained by the Municipality of Bombay for catching rats. Approximately 2,000 rats are received every day by the Institute from the different wards within the Municipal area. These rats, as in the previous years are either trapped alive or found dead in the various parts of the City including the Port Trust area. 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 infection in time.

Tables giving the monthly totals of rats received and the number out of them that were examined are given below. During the year under report, 6 plague infected rats were detected.

TABLE I—*Showing Rats received at the Institute from the Bombay Municipality during 1949*

Month	Alive	Dead	Total
January	58,041	49,942	1,07,983
February	56,088	30,605	86,693
March	[60,120	11,390	71,510
April	[80,472	8,699	89,171
May	[36,114	3,149	39,263
June	[4,341	380	4,721
July	16,563	4,501	21,064
August	25,877	6,834	32,711
September	33,850	10,374	44,224
October	47,460	8,998	56,458
November	76,091	7,847	83,938
December	84,222	15,018	99,240
Total	5,79,239	1,57,737	7,36,976

TABLE II—Showing dead rats examined for plague during 1949.

Month			Dead rats examined	Found plague-infected <i>Rattus</i> <i>rufescens</i>	<i>Gunomys</i> <i>kok</i>
January	..	..	18,223	Nil.	Nil.
February	..	..	11,707		1
March	..	..	2,508	3	1
April	..	..	2,420	1	Nil.
May	..	..	818	Nil.	
June	..	..	293		
July	..	..	726		
August	..	..	485		
September	..	..	1,722		
October	..	..	1,084		
November	..	..	1,723		
December	..	..	7,208		
Total			.. 48,917*	4	2

*In addition to dead rats, 191 live rats from the plague-infected localities and 6,985 live rats from the Bombay Port Trust area were examined during the year. The total number of live and dead rats examined is 56,093.

TABLE III—Showing the number and percentage of different species

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i>	11,872	26.3	9,967	21.7	9,920	21.6	15,085	24.0	7,642	25.6	1,293	35.3
<i>Rattus norvegicus</i> ..	8,944	19.8	11,028	24.0	10,223	22.3	13,101	20.9	6,247	20.9	689	18.7
<i>Gnomys kok</i> ..	17,691	39.1	19,155	41.7	18,873	41.0	24,820	39.6	11,579	38.7	1,188	32.3
<i>Bandicoota malabarica</i>	200	0.5	158	0.3	158	0.3	180	0.3	45	0.1	5	0.1
<i>Mus debius</i> ..	2,589	5.7	2,067	4.5	2,767	6.0	3,758	6.0	1,758	5.9	97	2.6
<i>Suncus caeruleus</i> ..	3,906	8.6	3,592	7.8	3,973	8.7	5,762	9.2	2,627	8.8	406	11.1
Total † ..	45,202	100.0	45,967	100.0	45,919	100.0	62,706	100.0	29,898	100.0	3,678	100.0

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

†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	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i>	3,929	10.2	2,539	10.0	698	7.6	717	10.7	301	11.3	117	38.2
<i>Rattus norvegicus</i> ..	4,242	11.0	2,757	10.8	580	6.4	426	6.3	144	5.4	55	18.0
<i>Gnomys kok</i> ..	8,417	21.9	5,865	21.1	1,066	11.7	823	12.3	352	13.3	66	21.6
<i>Bandicoota malabarica</i>	11	0.3	24	0.1	10	0.1	11	0.2	..	..	1	0.3
<i>Mus debius</i> ..	1,524	3.9	952	3.	87	1.0	39	0.6	8	0.3	2	0.7
<i>Suncus caeruleus</i> ..	2,102	5.5	1,284	5.1	87	1.0	56	0.8	6	0.2	..	..
Putrid	18,102	47.2	12,523	49.2	6,591	72.2	4,639	69.1	1,843	69.5	65	21.2
*Total ..	38,327	100.0	25,444	100.0	9,117	100.0	6,711	100.0	2,654	100.0	308	100.0

*These totals do not agree with the totals of dead rats in Table

use rats received on Sundays

of rats trapped alive by Municipal Corporation, Bombay, during 1949*

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
8,504	26.6	4,366	22.4	5,757	21.2	8,188	23.8	14,371	22.8	15,224	21.7	1,07,189	23.8
2,242	17.0	3,622	18.6	5,608	20.7	6,890	20.0	11,480	18.2	12,379	17.6	92,458	20.0
5,743	48.5	9,332	48.0	12,749	47.0	14,629	42.6	27,399	43.4	31,206	44.5	1,94,364	42.2
35	0.3	47	0.2	68	0.2	117	0.3	393	0.6	622	0.9	2,023	0.4
455	3.4	534	2.8	936	3.5	1,389	4.0	2,967	4.7	3,749	5.3	23,066	5.0
1,220	9.2	1,564	8.0	2,003	7.4	3,197	9.3	6,499	10.3	6,993	10.0	41,747	9.1
13,199	100.0	19,465	100.0	27,121	100.0	34,410	100.0	63,109	100.0	70,178	100.0	4,60,852	100.0

Institute dead, often mutilated and even putrid.

and holidays were not classified.

Species of rats found dead in the City of Bombay during 1949

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
185	5.0	95	1.9	331	4.0	311	4.9	574	8.6	1,481	11.9	11,276	9.0
117	3.1	101	2.0	389	4.8	204	3.2	429	6.5	1,602	12.9	11,046	8.9
418	11.2	275	5.4	969	11.8	529	8.3	691	10.5	3,811	30.6	22,782	18.2
4	0.1	2	0.0	1	0.0	4	0.0	3	0.0	25	0.2	96	0.0
1	0.0	15	0.3	32	0.4	34	0.6	26	0.4	283	2.3	3,009	2.4
33	0.9	8	0.2	89	1.0	72	1.1	123	1.9	609	4.9	4,469	3.6
2,966	79.7	4,575	90.2	6,399	78.0	5,226	81.9	4,772	72.1	4,630	37.2	72,331	57.9
3,724	100.0	5,071	100.0	8,210	100.0	6,380	100.0	8,818	100.0	8,119	100.0	4,60,852	100.0

B. EXAMINATION OF RATS FROM

These rats were received along with those of the other wards for in the monthly totals given in previous tables. A separate different species, is also maintained at the instance of the Public

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 rattu rufescens</i>	188	27.8	240	39.7	162	24.9	261	41.3	339	30.8	1,042	35.8
<i>Rattus norvegicus</i> ..	113	18.7	87	14.4	119	18.3	97	15.3	143	13.0	577	19.8
<i>Gunomys Kok</i> ..	262	43.3	174	28.8	261	40.0	173	27.3	471	42.8	861	29.6
<i>Bandicoota ma abarica</i>	7	1.1	1	0.2	3	0.4	2	0.3	1	0.0	5	0.2
<i>Mus dubius</i> ..	12	2.0	41	6.8	17	2.6	46	7.3	48	4.4	76	2.6
<i>Suncus caeruleus</i> ..	43	7.1	61	10.1	90	13.8	54	8.5	99	9.0	351	12.0
*Total ..	605	100.0	604	100.0	652	100.0	633	100.0	1,101	100.0	2,912	100.0

* Rats received on Sundays and holidays were not classified.

THE BOMBAY PORT TRUST AREA

of the Bombay Municipality, and have been already accounted record of these rats, giving the number and percentage of the Health Commissioner. Table V gives summaries of the records.

of rats caught alive from the Bombay Port Trust Area in 1949

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
872	40.1	310	40.5	290	40.2	270	43.1	367	53.7	344	39.8	4,665	37.8
278	12.8	104	13.6	126	17.5	89	14.2	80	11.7	112	13.0	1,925	15.6
724	33.3	242	31.6	208	28.8	201	32.0	162	23.7	341	39.5	4,080	33.0
11	0.5	..	..	1	0.1	..	..	2	0.3	5	0.5	38	0.3
54	2.5	35	4.3	24	3.3	7	1.1	14	2.1	15	1.7	387	3.2
236	10.8	76	10.0	73	10.1	60	9.6	58	8.5	47	5.5	1,248	10.1
2,175	100.0	765	100.0	722	100.0	627	100.0	683	100.0	864	100.0	12,343	100.0

C. EXAMINATION OF RATS FROM STEAMERS AND OTHER SOURCES

During the year 1949, 1,713 dead rats and 96 smears from dead rats were received from different towns of the Bombay Province. As a result of their examination 14 rats were reported upon as being plague-infected from towns mentioned in Table VI.

TABLE VI—*Showing the different localities in the Bombay Province from which plague-infected rats were received during 1949.*

Name of Town.				Number of plague-infected rats.
Ahmednagar	..	..	..	13
Dharangaon	..	..	..	1
				14

During the year under review, 491 killed rats from steamers were received from the Health Officer of the Port of Bombay and of these 292 belonged to the species *Rattus rattus rufescens*, 12 *Gunomys kok* and 187 *Mus dubius*. 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 places in the Province. In addition, private medical practitioners contributed a good number of samples for diagnostic work. Free diagnostic aid was given to all practitioners in infectious diseases such as diphtheria, dysentery, cerebro-spinal fever, enteric fever, typhus fevers, cholera, plague, malaria, etc. The typhoid scheme sanctioned by Government in December 1947, continued its operation during the year 1949 and the Institute medical officer attended 795 calls from private practitioners. The scheme is becoming very popular, as larger number of medical practitioners are taking advantage, primarily because early diagnosis helps detection and notification of this disease with timely measures against its spread.

The following table gives an idea of some of the important examinations carried out during the year under report:—

Nature of examination				No. of samples examined	No. of positive samples
Wasserman test	..	..	..	7,461	1,799
Kahn test	..	..	..	2,079	377
Widal test	..	..	..	2,758	1,020
Agglutination test for typhus fever			..	2,810	135
Agglutination test for Brucellosis			..	2,769	7
Material for T.B. by smear, culture and animal inoculation test.				450	65
Throat swabs for diphtheria	..	..	..	74	20
Stools for routine Exam.	..	..	..	688	139
Stools for culture (including rectal swabs)	..	..	..	1,316	54
Blood films for malaria	..	..	..	884	25
Material for plague	..	..	..	40	5
Friedman test and Rat test for pregnancy	..	..	..	253	104
Urine for cultural examination	..	..	..	185	44
Urine for routine examination	..	..	..	548	
C. S. F.—routine and culture	..	..	..	268	
Blood counts	..	..	..	811	
Material for detection of gonococci	..	..	..	70	
Material from medico-legal cases	..	..	..	42	
Rideal Walker test	..	..	..	63	
Autogenous vaccines	..	..	..	43	
Semen examination	..	..	..	50	

The City was practically free from cholera during the year, although fifteen strains were isolated from cases which were all imported. Five cases of plague were definitely established by laboratory examination and they were all imported. Out of 36 strains of dysentery organism that were isolated, 28 belonged to *B. flexner* type, six to *B. shiga* type and one to *B. schmitzi*. One hundred and sixty-eight cases were also

This section was also engaged in the maintenance of various species of poisonous snakes for the purpose of extraction of venom. The venom was quickly freeze-dried and maintained in vacuum sealed ampoules at refrigeration temperature. The quantities of different venoms collected during the year under report are given below :—

Year.		Cobra venom in gms.	R. viper venom in gms.	Krait venom in gms.	Echis venom in gms.
1949	..	.. 22.899	24.821	2.768	12.241

The diagnostic reagents section carried on the work of preparing reagents for diagnostic purposes. The reagents are supplied to laboratories all over India and the demands are increasing every year. The following reagents were supplied during the year under report :—

Reagents.	Amount supplied.
1. Blood grouping sera	3,188 ml.
2. High titre sera	308 ml.
3. Killed suspensions for Widal and Weil-Felix tests	1,07,917 ml.
4. Kahn antigen	1,020 ml.
5. Amboceptor	49 ml.
6. J. S. B. stain	10 lbs.
7. W. R. antigen	315 ml.

B. SECTION OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY

This Section mainly examined samples of waters for their fitness for drinking and industrial purposes and samples of aerated waters for fitness for human consumption.

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

Sample examined.	Nature of examination.	Purpose of examination.	Number of samples examined.
Water	Bacteriological ..	For fitness for drinking purposes.	50
Water	Bacteriological ..	For fitness for swimming purposes.	7
Water	Chemical ..	For fitness for drinking and sanitary purposes.	22
Water	Chemical ..	For fitness for industrial purposes.	9
Water (aerated)	Bacteriological ..	For fitness for human consumption.	7
Extract of Cola	Bacteriological ..	For fitness for use in aerated waters.	1

VII. Department of Pharmacology

After the introduction of the Drugs Act, 1940, and the Rules thereof, one of the chief functions of this Department has been to assay and test chemicals, drugs, biological products on behalf of the Government of Bombay. Many medicinal substances that were imported into this country through Bombay Customs were also examined in this Laboratory. Besides these several drugs manufacturing firms took advantage of the facilities offered at this Institute for testing of their products.

1. *Examination of drugs, chemicals, etc., under the Drugs Act, 1940, and the Rules thereof.*—The number of samples received from the Drugs Controller for the Province of Bombay reached a total of 340. Majority of these drugs were of pharmacopoeial preparations. Out of 259 samples analysed about 50 per cent. were found to be of standard quality and 46 per cent. were below standard. Out of the eight samples of calcium gluconate examined here only three conformed to the B. P. specifications. Six samples of Vinum Ipecacuanhana were tested, but none of them could be certified as of standard quality. In fifty per cent. samples of quinine bihydrochloride, the alkaloidal content was less than the claim made by the manufacturer. One sample of Menthol (received from the Drugs Inspector, Delhi) contained 98.0 per cent. of non-volatile matter whereas B. P. allows only 2 per cent. as the maximal limit. There cannot, therefore, be any doubt about the serious situation regarding adulteration of drugs in this country.

2. *Analysis of Drugs under Rule 40 of the Drugs Rules, 1945.*—During the period under review, 95 medicinal preparations which were imported into this country were received from the Collector of Customs, Bombay. About 92 per cent. of these samples were found to be of standard quality.

3. *Analysis of Drugs under Schedule C and C(1).*—Samples of Digitalis, Adrenaline, Pituitary, Vitamins, Insulin, etc., were received in this Laboratory both from the Drugs Controller for the Province of Bombay as well as from manufacturing firms. Out of the eleven samples of vitamin B₁ that were tested only five of them declared to be of standard quality. The quality of the vitamin C in the Indian market was better, for only one out of five samples examined did not comply with the B. P. specifications.

4. *Testing of injectable solution for the absence of Pyrogen.*—Samples of distilled water, glucose solution, normal saline, protein hydrolysate were examined for absence of any pyrogenic substances. The methods that were recommended in B. P. and U. S. P. were followed. The test involves use of rabbits and recording their rectal temperature. The total number of samples examined were 95 of which 61 were from the Haffkine Institute. Only five samples failed to pass the pyrogen test.

6. *Commercial Testing of Drugs.*—Some of the drug manufacturing firms who do not maintain separate analytical laboratory or wanted their medicines to be examined in a Government Laboratory had their products tested on payment of prescribed fees. More than fifty samples were analysed here.

7. *Preparation of Sulphanilamide containing Vitaminised Oil.*—This Department continued to make sulphanilamide paste for the purpose of distributing to various hospitals. About 537 pounds of the paste were manufactured here during 1949.

VIII. Department of Chemotherapy

The regular manufacture of sulphathiazole was undertaken. As a result of the experience gained during the earlier days, a certain amount of realignment of machinery for more facile working had to be done. In addition, many improvements in the processes, involving reduction of costs and easier working methods, have been effected. The designs of the auxiliary equipment fabricated here and purchased, and also the other details of the set-up were worked out by the workers of the department themselves without any outside help. The production records indicate that during the period when regular batches were run, the production reached the quantity set up as the target, though originally, the plant was designed for just half the capacity only. During the year under review, about 2,000 lbs. of sulphathiazole and 3,500 ampoules of the injectible sodium sulphathiazole solution were manufactured. The sulphathiazole tablets as well as the injectible prepared were regularly supplied to the Government hospitals, dispensaries, public health institutions and also to charitable institutions. Besides, as required by the Public Health Department, the department has also built up a stock of sulphathiazole as a precaution against the possible outbreak of influenza. The process of manufacture of sulphathiazole is now well standardised to run on routine lines.

Attention is now being directed towards the large scale preparation of the other very important and useful drug, sulphamerazine. Very exhaustive investigations on the various processes available for the production of this drug on a commercial scale are being carried out. One process which is adaptable to our conditions and equipment on hand, has been worked out and this is being investigated in detail to take it up on a pilot plant scale. It is hoped that the production of sulphamerazine will be on routine lines during the ensuing year.

When the Government wanted to know our most probable cost of production of paludrine, about which there was some serious difference of opinion, a number of experimental batches on plant scale were run to gather data under the various headings required by the auditors to enable them to calculate our cost of production. As a result of this, the original cost arrived at by us (Rs. 20 per pound) was confirmed. The production could not be undertaken since the process is covered

by a patent held by the I. C. I. and the licence question has not been settled. Parallel to this, investigations have been undertaken to develop other methods of production of the drug and the results so obtained show some promise.

About 2,000 lbs. of the antiseptic solution "Pemon" were prepared and supplied. Phenyl mercuric nitrate which is being used as a preservative in the vaccines manufactured at the Institute was also prepared.

Under instructions from the Government, the department has undertaken to prepare and supply the drugs now being used for the treatment of leprosy. Hydnocreol oil was processed, ampouled and supplied to leprosy clinics. The preparation of "Sulphetron injectible" is being undertaken.

The microanalytical laboratory is now fully equipped and standardised for undertaking the analysis of new organic compounds prepared in connection with the research work.

The research work carried out by the department was directed towards the chemotherapy of leprosy, tuberculosis and malaria. A large number of compounds of various types (sulphanilamides, sulphor thiazoles, quinolines, guanidines and biguanides) have been prepared. The compounds prepared as possible antimalarials are tested for their antimalarial activity. A detailed account of these is being given in the research section.

VITAMIN TABLETS SECTION.

This Section works on two lines : (1) The production of Vitamin A Concentrate from the Shark-liver Oil and (2) The manufacture of vitamin tablets and sulphathiazole tablets.

THE CONCENTRATE SECTION.

4,840 lbs. of Shark-liver oil were processed during the year under Concentrates of average potency of 2,50,000 I. U. per Gm. were

Experiments were carried out with a view to stabilise the Vitamin A Concentrate and increase its shelf life, as well as with a view to extend the period of its potency when incorporated in tablets. The experiments are still underway but the preliminary results appear to be encouraging.

THE TABLETTING SECTION.

This section continued the manufacture of vitamin and sulphathiazole tablets as usual. At the beginning of the year, to work out the cost, the preparation of Paludrine hydrochloride tablets was carried on an experimental scale. In the next two months, the section was busy to its fullest capacity with the manufacture of about four million

The best compositions for the manufacture of the individual vitamin tablets such as Vitamin B1, Vitamin B2, Vitamin C and Acid Nicotinic Tablets were well established in order to keep the cost of these tablets as low as possible, consistent with the high standard of the product. The demand for these tablets is daily increasing as can be seen from the comparative figures of production for the last year and the year under report.

The manufacture of sulphathiazole tablets was successfully handled and we have enough stocks to meet demands from the Government hospitals, dispensaries and other Government approved bodies.

The number of tablets prepared are given in the following table :

Kind of Tablets			Production during 1948	Production during 1949
1.	Vitamin A-D tablets		7,05,470	57,82,620*
2.	Vitamin B-C tablets		7,35,030	57,91,010*
3.	Vitamin B1 tablets		1,73,845	2,44,150
4.	Vitamin B2 tablets		36,900	69,290
5.	Vitamin C tablets		1,99,800	4,11,300
6.	Acid Nicotinic Tablets		16,320	1,65,450
7.	Sulphathiazole Tablets		1,08,000	8,46,655

*Out of these approximately 4 million of each were prepared for the Government for free distribution to the famine-stricken area.

During the course of the year it was noticed that inspite of the competitive prices of our products, a very small fraction of the public was at all aware of the availability of this excellent facility at this Institute. It is hoped that with due publicity, the benefit of this facility would be extended to a large section of the public.

A number of enquiries were received for vitamin injectibles. The matter of taking up the manufacture of vitamin injectibles as well as the sale of vitamin tablets to the public through Government approved dealers, is under the consideration of Government.

IX. Department of Biochemistry

During the year under report as in previous years this department gave useful aid to hospitals and practitioners by carrying out the various routine biochemical examinations necessary for the diagnosis of diseases.

The number of samples examined shows a steady increase, the total examinations for the year being 2,935. This comprises, blood examinations 1,704, urine 339, cerebro-spinal fluid 625, body fluids 90, special tests such as gastric analysis 36, glucose tolerance tests 60, blood urea clearance tests 36, basal metabolic rate determinations 43 and faeces analysis one. The routine examinations were mostly determinations of non-protein nitrogenous constituents, such as, urea, uric acid, serum proteins, and enzymes such as phosphatase, lipase amylase, diastase, and inorganic constituents such as calcium, sodium, phosphorus etc. Duplicate samples of milk, collected at different distribution centres were analysed, the total number being 203.

In addition to the routine work, a critical study of the inter-relationship between niacin and tryptophane in the metabolism of the rat has been undertaken. As a preliminary to this, a comparative study of the assay of niacin by chemical and microbiological methods using *Lactobacillus arabinosus* 17/5 was carried out and it was found that the specific microbiological method was most suitable for the present investigation. For the basal medium in the microbiological assay Casein hydrolysate as well as peptone solution treated with charcoal were tried and the former was found to give much more variable results.

Knowledge concerning the role tryptophane plays in the metabolism of niacin is still not conclusive. Conflicting experimental data have been obtained regarding the actual site of synthesis of niacin from tryptophane. The present study has been undertaken to investigate this aspect of the problem and also to study the distribution of niacin in the different organs of the body of rats fed with diet deficient in niacin but supplemented with tryptophane.

X. Department of Nutrition and Experimental Pathology

A. NUTRITION SECTION

The Department continued to render useful service as in previous years, to the Civil Supplies Department of the Government of Bombay. During the year 1949 about 110 different samples of foodstuff 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, 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. Most of the foodstuffs received from the Government were analysed with a view to find out their fitness or otherwise for human consumption.

Statement showing the number of Pathological tissues received and examined at the Institute during the year 1949—contd.

Haemangioma	..	..	..	..	..	5
Lipoma	..	..	..	..	..	1
Mycetoma	..	..	..	..	..	1
Neurofibroma	..	..	..	..	..	1
Osteofibroma	..	..	..	..	..	1
Papillary cystadenoma	..	..	..	..	..	1
Papilloma	..	..	..	..	..	2
Polypus	..	..	..	..	..	33
Pseudomucinous cystadenoma	..	..	..	..	..	9
Serous cystadenoma	..	..	..	..	..	3
(c) <i>Mixed Parotid Tumour</i>	..	..	..	..	..	1
(d) <i>Rhinosporidiosis</i>	..	..	..	..	..	1
(e) <i>Haemangio-endothelioma</i>	..	..	..	..	..	2
(f) <i>Teratoma</i>	..	..	..	..	..	2
II. TUBERCULOSIS						
Breast	..	..	..	..	..	2
Chest	..	..	..	..	..	1
Female Genital tract	..	..	..	..	..	18
Foot	..	..	..	..	..	1
Lymphnodes	..	..	..	..	..	15
Knee joint	..	..	..	..	..	1
Larynx	..	..	..	..	..	1
III. RARE GYNAECOLOGICAL CONDITIONS						
Cyst of the broad ligament	..	..	..	..	..	1
Cyst of Bartholin gland	..	..	..	..	..	1
Dermoid cyst	..	..	..	..	..	3
Follicular cyst of the ovary	..	..	..	..	..	2
Lutein cyst of the ovary	..	..	..	..	..	2
Granulosa-lutein cyst of the ovary	..	..	..	..	..	1
Pseudomucinous cyst of the ovary	..	..	..	..	..	1
Serous cyst of the ovary	..	..	..	..	..	1

Statement showing the number of Pathological tissues received and examined at the Institute during the year 1949—contd.

IV. MISCELLANEOUS PATHOLOGICAL CONDITIONS

Miscellaneous pathological conditions	..	..	..	440
Tissues from Experimental Animals	..	..	..	330
V. TISSUES FOUND TO BE NORMAL ON HISTOLOGICAL EXAMINATION	..	..	..	42
VI. TISSUES RECEIVED IN A STATE UNFIT FOR EXAMINATION				66
VII. BRAINS OF ANIMALS FOR EXAMINATION OF NEGRI BODIES	..	..	..	118
Total				1,262

Some interesting specimens received during the year are given below :—

Linitis plastica of the stomach ; Fibrous epulis of the jaw ; Colloid carcinoma of the rectum ; Malignant papillary cystadenoma of the ovary ; Cystic hygroma of the neck ; Mycetoma of the Foot and Malignant Melanoma of the Foot.

During the months of September to December 1949, Dr. Prithviraj Gambhir, Clinical Pathologist, General Hospital, Udaipur (Rajasthan) was given training in histopathological technique and morbid histology.

Dr. C. V. Natarajan (Mysore) Rockefeller Foundation Scholar, paid a short visit to the Department in the month of September, to study the experimental production of hepatic cirrhosis by dietary deficiencies.

XI. Department of Virus Diseases including Rabies

This department continued to (a) ; afford anti-rabic treatment to patients in the northern part of the island of Bombay (b) prepare very large quantities of anti-rabic vaccine for issue to anti-rabic treatment centres ; (c) assist in diagnosis of rabies in animals by histological examination of brain tissues ; (d) hold stocks of Yellow Fever Vaccine and conduct inoculations with the assistance of the special staff of the Yellow Fever Diagnostic Unit sanctioned by the Government of India and (e) conduct reasearch in virus diseases.

(a) *Anti-rabic Treatment Centre at this Institute.*—During the year under report, 5,413 persons applied for anti-rabic treatment. Of these 4,620 completed the full course of treatment, 775 were advice cases and the remainder 18 discontinued treatment. In cases where there was a doubt about the condition of the dog inflicting the bite and the dog was available for observation the patients were given two injections and were advised to keep the dog under observation for 10 days. At the end of this period of observation. if the dog

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

			Positive	Negative	Unfit	Total	Percentage
Bombay City	..	..	22	25	2	49	39.52
Suburbs	..	..	4		2	6	4.84
Outstations	..	..	24	41	4	69	55.64
			50	66	8	124	100.00

TABLE IV—*Table showing the number of brains received and examined at the Haffkine Institute for evidences of rabies during the year 1949*

Details		Number	Percentage
Positive for rabies (Negri bodies present)	..	50	40.32
Negative for rabies (Negri bodies absent)	..	66	53.23
Received in a state unfit for examination	..	8	86.45
Total	..	124	100.00

TABLE V—*Statement showing the classification of different animals with the results of their brain examination for rabies during the year 1949*

Animals			Positive	Negative	Unit	Total
Dog	..	..	47	61	7	115
Goat	..	..	3	2		5
Bullock	..	..		2		2
Cow	..	..		1		1
Fox	..	..			1	1
			50	66	8	124

(d) *Yellow Fever Vaccine and Vaccination.*—The Yellow Fever Diagnostic Unit of the Haffkine Institute continued its work of Yellow Fever Inoculations and testing of the potency of Yellow Fever Vaccine stocked on behalf of the Government of India. 19 batches of the Rockefeller Foundation Yellow Fever Vaccine were tested for potency of their Virus content during the year. The number of tests performed were 51. Repeated potency tests on several batches have shown that the potency of the vaccine is retained much longer than one year. Some batches have, so far, been found to be potent for even three years or more.

The number of persons immunized against Yellow Fever during 1949 are shown in Table VI. These persons were air or sea passengers from Bombay proceeding to or passing through Yellow Fever endemic areas. The figures also include the staff of Civil Hospitals and infectious diseases hospitals in Bombay Province, who have been earmarked for treating Yellow Fever Patients in case of an outbreak of Yellow Fever in Bombay Province.

Month						Number of inoculations against Yellow Fever
January	..	..	..	..	..	384
February	..	..	..	..	..	802
March	..	..	..	..	..	563
April	..	..	..	..	..	1,356
May	..	..	..	..	..	651
June	..	..	..	..	..	618
July	..	..	..	..	..	457
August	..	..	..	..	..	801
September	..	..	..	..	..	592
October	..	..	..	..	..	427
November	..	..	..	..	..	585
December	..	..	..	..	..	649

Yellow Fever Vaccine stored at the Haffkine Institute was distributed during the year to the following Yellow Fever Inoculation Centres.

Serial No.	Centre	Quantity of vaccine supplied in doses
1	King Institute, Guindy, Madras	2,000
2	Provincial Public Health Laboratory, New Delhi ..	1,600
3	Chief Medical Officer, Porbunder	500
4	Port Health Officer, Jamnagar	500
5	Director General of Medical Services, Noa-foa ..	600
6	Medical Research Institute, Ceylon, Colombo ..	Nil.
7	Air Port Health Officer, Santa Cruz	360
8	All India Institute of Hygiene and Public Health, Calcutta..	Nil.
Total ..		5,560

(e) *Research*.—Random samples of Semple's Vaccine were assayed and found to possess a high degree of potency.

Studies on another Canine Vaccine, as well as the keeping qualities of Semple's Vaccine were continued.

XII. Blood Bank Department

During the year under report, the work of the department showed a considerable increase over the last year's work. The demand for dried plasma has been on the increase especially from the private hospitals and practitioners and Institutions from outside the province. The response of the voluntary donors has been as unsatisfactory as before. However, the number of volunteers from the prisoners has increased and since the Government has sanctioned the payment of Rs. 3 to each prisoner for each donation, this number has considerably increased and this has helped us in meeting the increased demand of plasma. The Surgeon General has allotted free quota of plasma to public hospitals in the City and mofussils as before; but some of the City hospitals are not taking full advantage of it.

A new ambulance van has been provided for the mobile Blood Collecting Unit fitted up with refrigerators and this will facilitate the working of this unit greatly.

Dr. T. D. Patel, the Medical Officer in charge Blood Processing Unit has proceeded abroad for study tour in Blood Bank technique to U. S. A. and U. K. and the experience he gains there will be of great value to the working of the department.

As usual all the blood samples collected were grouped and Kahn test was performed. The donors were screened for their fitness by copper sulphate sp. gr. method. The following is the account of the work done.

Three thousand four hundred and thirteen samples were examined by Kahn test and of these 545 samples were found Kahn positive.

Five thousand four hundred ml. of blood with high titre of haemagglutinins was supplied to the Diagnostic Department for preparation of Blood grouping sera.

Seven hundred and fifty two blood donors were screened by copper sulphate sp. gr. method for fitness to give blood, of which 222 were rejected.

Seven thousand and fifty six ampoules of Antivenene were dried.

The new batches of housemen appointed at J. J. Group of Hospitals were deputed to the Institute for training in Blood Transfusion Technique.

The following statement shows the progress of work done in this department during the year 1949.

TABLE NO. I—*Showing the Number of Blood Donors.*

Centre	Type of donor				Number of blood donors
Haffkine Institute ..	..	Voluntary	..	..	3
	..	Paid	..	..	529
	..	Jail Donors	..	..	1,709
		Total	..		2,241
Indian Red Cross Centre	..	Voluntary	..	..	48
	..	Paid	..	..	1,080
		Total	..		1,128

TABLE NO. II—*Showing the Blood and Plasma yield*

Product.						Quantity in litres.
Blood	..	..	..	..	..	8,40,120
Liquid Plasma	..	..	..	..	..	4,59,500
Dried Plasma	..	..	..	..	..	5,10,000

TABLE III—*Showing the distribution of dried Plasma*

Nature.						Quantity in litres.
Free	..	..	..	..	..	89,500
Paid	..	..	..	..	..	3,30,250
Total						4,19,750

The work of Rh factor was handicapped for shortage of standard testing serum. Recently some standard serum has been received from U. S. A. and now efforts will be made to detect and to collect sera from suitable cases. This work will be done in co-operation with the City hospitals.

As suggested by the Scientific Advisory Board of the Indian Research Fund Association, the collection and distribution of Anti Rh sera will be undertaken by this Institute for this province.

The Honourable Minister of Health had proposed preliminary work in connection with medico-legal serology be done in the Institute with a view to the establishment of the full fledged Serological Department. The work was undertaken in co-operation with the Chemical Analyser and already potent Antihuman precipitation sera have been prepared and Anti M and Anti N sera and Anti Rh sera are also being prepared. Also other techniques in connection with medico-legal serological work are developed.

XIII. Training of Health Personnel

The Institute plays an important part in the dissemination of scientific knowledge by affording facilities to persons desirous of learning different types of techniques either for the prosecution of research 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 disease control work efficiently. Usually most of the workers are from this country. The names of the persons who received such training with the periods of their training and the department in which they worked are given in the following table.

Sl. No.	Name	Designation and Address	Department in which trained	Duration of training
1	Mr. Mir Habibullah, M. Sc.	Chemist, Drugs Research Laboratory, Jammu and Kashmir State, Jammu-Tawi.	Vaccines, Antitoxins and Sera, Chemotherapy and Nutrition.	17th January 1949 to 28th February 1949.
2	Dr. J. D. Kohli, L.T.M., L.S.M.F.	Assistant Bacteriologist, Drugs Research Laboratory, Jammu and Kashmir State, Jammu-Tawi.	Vaccines, Antitoxins and Sera, Clinical Pathology and Media.	23rd January 1949 to 6th May 1949.
3	Mr. V. H. Patel, B. Pharm	Demonstrator, L. M. College of Pharmacy, Ahmedabad, 6.	Biochemistry and Pharmacology.	22nd March 1949 to 28th May 1949.
4	Mr. K. Velayudhan Pillay, B. Sc.	Bacteriological Assistant, Public Health Laboratory, Trivandrum.	Clinical Pathology, Virus Diseases including Rabies, Vaccines, Chemotherapy and Animal House.	2nd April 1949 to 30th April 1949.
5	Major M. Dutta, I. A. M. C.	Bacteriologist, Pathology Department, Armed Forces Medical College, Poona.	Animal House.	26th April 1949 to 28th April 1949.
6	Dr. S. R. Patwardhan	Medical Officer, The Tata Power Co., Ltd., Camp Bhira, P. O. Kolad, District Kolaba.	Virus Diseases including Rabies.	16th May 1949 to 22nd May 1949.
7	Dr. A. S. Dikshit	Medical Officer I/c Vaccine Section, Provincial Hygiene Institute, U. P., Lucknow.	Vaccines.	21st June 1949 to 5th July 1949.
8	Dr. Prithiviraj Gambhir, L.M.S., D. T. M.	From the Inspector-General of Hospitals and Prisons, United State of Rajasthan, Udaipur.	Clinical Pathology and Experimental Pathology.	4th July 1949 to 20th December 1949.
9	Dr. C. V. Natarajan, M. B. B. S., D.P.H., F. R. I. C., F.A. Sc.	Director of Public Health Laboratory, Bangalore.	Vaccines and Antitoxins and Sera.	29th August 1949 to 7th September 1949.
10	Mr. M. J. Gulrajani, B.Sc.	Water Analysis Laboratory, The Sindhu Resettlement Corporation Ltd., Karimji House Sir P. M. Road, Bombay.	Section of Public Health Chemistry and Bacteriology.	December, 1949.

XIV. Critics Club

The activities of the Critics Club continued as before. Weekly meetings were held every Friday, in which current literature on scientific subjects was reviewed by the members of a department, and once a month a brief account of the original researches was given by a member.

During the year, 47 meetings were held, at which abstracts of current literature pertaining to various subjects were read by the members of different departments, and the members were addressed twice by distinguished guests Sir C. V. Raman of Bangalore and Prof. J. D. Bernal of Birbeck College, University of London. Following films on interesting scientific subjects were shown by the Imperial Chemical Industries, United States Information Service and Lederle Laboratories:—

1. (a) Fight against Disease (b) Story of Penicillin (c) Extraction of Penicillin (d) Physiology of Kidney (e) Perfusion of the Living Animal—by Imperial Chemical Industries on 2nd May 1949.
2. "University of California" by U. S. Information Service on 2nd September 1949.
3. "Plague" (a) Methods of prevention (b) Tissue culture of White Cells (c) Tissue Culture of Fibroblasts—by Lederle Laboratories on 7th September 1949.

Individual talks were given by the following workers on the subjects mentioned below:—

1. "Talk on Miracles" by Dr. J. P. Menezes on 28th January 1949.
2. "Autoblood Penicillin Mixture for Treatment of Gonorrhoea" by Dr. B. G. Modi on 25th March 1949.
3. "Aureomycin" by Mr. M. K. Habbu on 29th April 1949.
4. "The Lysenko Controversy" by Dr. K. Ganapathi on 27th May 1949.
5. "Recent Knowledge of the Epidemiology of Poliomyelitis" by Dr. D. W. Soman on 29th July 1949.

XV. Income and Expenditure

The following table shows the income and expenditure of the Haffkine Institute for the year 1948-49.

Receipts.		Expenditure.	
	Rs.		Rs.
XXVII—Medical—		38—Medical—	
Sale Proceeds of Sera, Vaccines and Miscellaneous Receipts.	24,12,072	H—Bacteriological Laboratories—	
		H-1 Haffkine Institute,	
		H-2 Pasteur Institute.	
		Pay of Officers ..	1,75,928
		Pay of Establishment ..	3,97,178
		Allowances, etc. ..	2,75,067
		Contingencies ..	10,20,296
Total Receipts ..	24,12,072	Total Expenditure ..	18,68,469

PART II**RESEARCH WORK.****I. Aureomycin and Chloromycetin in the Treatment of Plague
Experimental Infection**

(SOKHEY AND HABBU)

Whenever any new drug likely to have a curative action against human plague infection is available, it is our practice to test its value in experimental plague infection before trying it in the field in human plague cases. Since plague is primarily a disease of rodents it is possible to produce natural infection in experimental animals and it has been our experience that results of tests in experimental infection always parallel those of clinical trials.

The two new antibiotics, Aureomycin and Chloromycetin, which were effective against gram negative organisms and therefore supposed to possess antibiotic effects against plague organisms were tested for their curative value in experimental plague infection in mice.

The most important factor which decides the issue in both experimental and human infection is development of septicaemia. In experimental animals, cent per cent animals die once the septicaemia is developed. If therefore a drug is given after the septicaemia develops it gives a much more accurate idea about the curative value of the drug than if the drug is given before the septicaemia sets in. The administration of these drugs was, therefore, started after septicaemia had developed in mice, i.e. 72 hours after the induction of infection. The drugs were emulsified in 10 per cent aqueous solution of gum acacia so that 0.5 c.c. contained the selected doses. This quantity was given by mouth every 6 hours for 5 to 10 days. Aureomycin was given in a dose of 1.0 mgs. every 3 hours during the first 12 hours, and then every 6 hours for the rest of the 10 days. That is, in all, a quantity of 42 mgs. was given and this saved 9 out of 10 mice. In the case of Chloromycetin a dose of 8 mgs. every 3 hours during the first 12 hours and then every 6 hours for the rest of the 10 days, i.e. in all 336 mgs. was necessary to obtain

in sufficient quantity or for a sufficient number of days, infection re-appeared after an apparent cure for some days. The results obtained are summarised in the following table :

Table.

Drug.	Dose of the drug.	No. of organisms in the Test Infe-ctive Dose.	No. of mice used.	Deaths during the period of admin-istration of the drug.	Deaths after the drug was stopped.	Controls	
						Mice used.	Deaths.
Aureomycin.	1.0 mg. four times a day for 10 days but the same dose given every 3 hours during the first 12 hours.	98	10	1	0	10	10
Chloromycetin.	8.0 mgs. four times a day for 10 days but the same dose given every 3 hours during the first 12 hours.	150	10	1	0	10	10

II. Glycerin and Rhamnose Fermentation by *P. Pestis*

(WAGLE AND HABBU)

Kaswashima (1934) reported that *P. pestis* could be classified into 3 groups on the basis of glycerin and rhamnose fermentations, viz. (1) strains which acidified neither glycerin nor rhamnose (2) those which produced acid in both cases (3) and those produced acid in glycerin medium alone. Chen (1949) who studied the behaviour of 53 strains of *P. pestis* from the collection of the George William Hooper Foundation, reported a fourth group which acidified rhamnose but produced no change in glycerin medium.

All these fermentation reactions are of value only if these reactions remain stable.

Francis (1932) when re-testing a strain isolated in 1923 from four different sources reported that he found no change in fermentation reactions. Kuaruchi (1936) confirmed the findings of Francis. But Korobkava (1936) reported that strains which originally did not ferment glycerin, when kept for a period of 10-12 years and then tested fermented glycerin. D'Aunoy (1923) also reported a similar finding.

We were, therefore, interested in knowing how the Indian strains behaved with regard to the fermentation of glycerin and rhamnose. The object of this study was to see if the different types of reactions exhibited by the strains could be correlated to the different localities from which they were obtained. We, therefore, selected 12 Indian strains from stock cultures, comprising of 3 freeze dried virulent cultures, 8 virulent cultures kept on blood agar and one avirulent strain kept on ordinary agar. All these strains were preserved for periods varying from 2 to 5 years in glass sealed tubes at 0°C without subculturing them during this period. We also included in this group 5 foreign strains viz. (1) Tjiwi smooth from Java (2) Tjiwidij rough from Java (3) E. V. from Macgaskar (4) Fukin A from China (5) Fukin B from China.

Of the Indian strains, 6 were from Hyderabad, one from Poona, one from Bombay, one from U.P. and 3 from Bettiah (Bihar). Employing essentially the same technique as that used by Chen we studied the behaviour of these 12 Indian and 5 foreign strains with regard to the fermentation of glycerin and rhamnose.

None of the strains tested fermented glycerin or rhamnose. All the strains were isolated and kept for more than two years, it was proposed to obtain freshly isolated strains from different parts of India and then study these reactions from time to time.

III. Inactivation of Cobra Venom with Soap Solution

(NIMBKAR AND LAHIRI)

Recent claims for efficacy of soap solutions against snake bite have been put forward by various workers. These claims deserve serious attention. With a view to test the efficacy of soap solution as a neutralizing agent against snake venom following work was undertaken :

Three commercial brands of soaps were tested :

1. Lifebuoy soap recommended by other workers.
2. A carbolic soap containing 6 per cent phenol and 30 per cent glycerine, and
3. An ordinary toilet soap containing no phenol. All the soaps were used in the form of 5 per cent soap solution.

Appropriate dilutions of solution of cobra venom in normal physiological saline were used.

Animals used were pigeons of 280-320 g. weight each, available in local market. Mice used were Laboratory inbred white mice of 20 g. weight each.

The neutralising power of soap solution was tested by two different methods (a) by mixing the venom and soap solution before the injection into animals and (b) secondly, by injecting the venom first followed by soap solution after a fixed interval of time. In pigeons, only the latter procedure was used. 3-5 mg of cobra venom were injected into the pectoral muscle of a pigeon. 2-5 ml of soap solution were then injected round about the site of injection of cobra venom after 5 minutes. Adequate control experiments were also carried out simultaneously. In addition to these controls two pigeons were also injected with four mg of cobra venom and subsequently were treated with a small quantity of Lyophilized anti-snake venom serum prepared by the Institute for comparison. All the animals were observed for 48 hours.

Similar tests were repeated on a much larger scale in white mice using both the procedures mentioned above. At first an optimum quantity of soap solution which the mouse could stand and the certain lethal dose of cobra venom for a mouse when injected subcutaneously were determined. Then the mixtures of varying quantities of cobra venom with an optimum quantity of soap solution and *vice versa* were prepared and injected into mice subcutaneously. All the animals were observed for 48 hours. Tests were carried out by the second procedure by injecting the venom first followed by soap solution after a fixed interval of time, using appropriate quantities of the venom and soap solution and varying the time between the injection of venom and soap solution.

The results showed that soap solution could not protect pigeons against lethal doses of cobra venom. In white mice, however, the soap solutions showed some protective power when large quantities of the latter were injected simultaneously with the venom. Injections of soap more than five minutes after the injection of venom failed to offer any significant protection. All the three brands of soap showed almost the same neutralising power. Moreover the small quantity of soap used caused intense necrosis of the tissue round about the site of injection. These considerations indicate the inadvisability of recommending soap solution for treatment of snake-bite in man.

IV. Typhoid Fever in the City of Bombay

(SOMAN AND MODI)

Free diagnostic aid in cases of typhoid fever continued in operation as before during the year 1949. The incidence of typhoid fever in Bombay rose again during the months of June, July, August and September, and the average monthly figures of the calls were as follows:

Year	Total	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1949	848	70	59	50	44	55	81	118	95	96	57	63	60

As a routine, blood was collected for Widal test, clot culture, blood culture, Weil-Felix test and agglutination test for brucellosis. Total and differential counts were done only when practitioners asked for the same. The number of samples tested serologically, definitely increased owing to greater publicity of the free facilities available at this Institute. Out of 2810 samples examined, which included 52 samples for Weil-Felix test only, 386 were diagnosed as of enteric and 135 were diagnosed as typhus. No case of paratyphoid B or paratyphoid C infection was diagnosed either serologically or bacteriologically. A feature of significant importance was the marked increase in the number of B paratyphus A strains isolated in relation to B.typhus. The ratio of B.typhus to B.paratyphus A strains was found 4:1 as compared with that of previous years which was 9:1. The following table illustrates the results for the year 1949.

Year	Total Samples	Hospital and Private	Typhoid Scheme	Widal+ Culture+	Widal— Culture+	Widal+ Culture—	Culture+	
							B. Typhoid	B. Para
1949	2,758	1963	795	331	277	689	493	115

Total number of calls registered was 848, of which 53 were such when in patients' address was incomplete or the patient refused to give blood. Thus 790 blood samples were collected and examined of which 386 were diagnosed as cases of enteric, of which 206 were bacteriologically positive. Out of 206 cultures, 46 were B. paratyphus A. As a routine, both blood culture (Hartley's Broth and clot culture Glycerine-Peptone Bile) were done on the same blood sample equally divided and hence comparative positive results obtained by clot and blood cultures were worked out. Out of 206, bacteriologically proved cases, both blood and clot cultures were positive in 116, only blood in 47 and only clot in 43.

Examination of stools and urine.—In spite of reporting serological or bacteriologically positive cases, very few samples of stools or urine were received either from hospitals or private practitioners to see whether the patients were excreting these organisms and did not act as carriers during or after convalescence.

Distribution of calls and enteric cases under typhoid scheme.—There were 750 calls from the City and 45 from the Suburbs and this distribution according to wards showed the following results:—

Wards	..	A	B	C	D	E	F	G	Suburbs	Total
Calls received	..	32	21	97	148	67	250	135	45	790
Enteric Cases	..	20	8	50	71	36	128	52	20	386

From the data available, it is found that Wards D, F and G show the highest incidence of enteric cases in the City for the year under report, although no account of private and hospital cases has been

V. Typhus in Bombay City and Suburbs

(SOMAN)

During the period under review, observations on typhus group of fevers were carried out as before and they were further extended to detection of Q fever in Bombay and its suburbs by serological methods. The specific Q fever antigen, strain and positive serum were obtained from three Laboratories in U.S.A. namely, Lederle Laboratories, Rocky Mountain Laboratories and National Institute of Health, Washington D. C.

Serological Diagnosis.—Sera from 2810 cases were examined for Weil-Felix test and 135 cases were diagnosed as belonging to typhus group of fevers. In these 135 cases, 114 cases were of proteus X19, 20 cases of proteus XK, and only one case of proteus X2. The history records of these cases did not reveal anything different from what was observed before.

Complement fixation test for the detection of Q fever antibodies.—The technique of complement fixation test was carried out as before using specific Q fever yolk-sac antigen, received from abroad and quite a large number of sera from febrile cases were tested. Out of 901 sera examined, 739 were sent for Widal test, 99 sent for W. R. and 43 were obtained from bandicoots caught in Poona ; the initial titres ranged from 1 : 10 to 1 : 80.

Only 3 sera from cases of undetermined pyrexia gave positive reaction in titres from 1 : 40 to 1 : 80. The tests when repeated failed to show any rise in titre, the positive reaction being ascribed to non-specific causes. Their clinical histories presented no resemblance to the clinical course of Q fever as observed in other countries. Sera from some of the bacteriologically proved typhoid or paratyphoid cases were found negative when tested and so also sera from typhus cases, X19 or XK in type. All sera from syphilitic and non-syphilitic cases and those obtained from bandicoots were found to be completely negative. Complement fixation tests were carried out since May 1949 to the end of the year. The tests were carried out using known positive and negative serum and other usual controls. Sera giving borderline reactions in titre of 1 : 40 or over were sent to Rocky Mountain Laboratories, Montana, U.S.A. for examination and the reactions were reported as non-specific. Further work is being continued.

Mite typhus.—Although sera from febrile cases were examined all the year round for presence of XK typhus, the same kind of seasonal prevalence, as reported before was observed during this year and only 20 cases of XK typhus with two deaths were diagnosed serologically. Attempts to isolate strains by mice inoculation were done in 15 cases

and the strains *R. orientalis* were successfully isolated in 6 cases. The following table illustrates the results :

Table

Widal No.	OXX Titre.	Day of fever.	Date of mice inoculation.	No. of passages.	Results.
2,400	200	14	11th November 1949	1st	<i>R. orientalis</i> .
2,464	100	10	19th November 1949	1st	„ „
2,490	1,600	..	24th November 1949	7th	„ „
2,664	400	17	15th December 1949	1st	„ „
2,733	200	19	23rd December 1949	5th	„ „
2,781	400	35	1st January 1950	5th	„ „

Cultivation of rickettsia in embryonated eggs.—A number of attempts were made to cultivate *R. orientalis* in the yolk-sac of a growing chick embryo but on account of the difficulties of getting a regular and adequate supply of fertile eggs, they were not successful beyond the first passage.

Search for the mite vector.—Total number of rodents examined for presence of mites were 220, out of which 89 were *Rattus rattus*, 85 *Rattus norvegicus*, and 46 were *Suncus caeruleus*. Only a small number were found to harbour mites ; thus total number of mites collected was 871, out of which 340 in 5 lots were used for inoculation into mice and the rest preserved for identification. It was not possible to carry out this work throughout the year or prior to the season of XK typhus cases. None of the inoculation experiments yielded any positive results inspite of repeated passages. Unless the problem of identifying the mite-vector of XK typhus in Bombay or its suburbs is tackled, the epidemiological studies will remain incomplete and hence the problem requires to be pursued with more vigour.

VI. Studies in the Synthesis of Sulphonamides

(M. V. SHIRSAT, K. D. KULKARNI, K. GANAPATHI)

Further work on the industrial application of the Indian Patent No. 39684 standing in the name of the Director, Haffkine Institute, has been conducted to investigate the various conditions for further improvements in the yields of sulphathiazole and consequent reduction in the cost of manufacture. A number of very encouraging observations

VII. Synthesis of Anti-tubercular Agents

(S. S. SABNIS, M. V. SHIRSAT, K. GANAPATHI)

Derivatives of para-amino salicylic acid are being synthesised with a view to obtain anti-tubercular agents of lesser toxicity and increased activity.

VIII. Studies in the Synthesis of Sulphonamides

(M. V. SHIRSAT, K. D. KULKARNI, K. GANAPATHI)

Further application of the chemistry disclosed in the Indian Patent No. 39684 with regard to its application to higher homologues of sulphathiazole are being studied. The method has been successfully applied to the higher homologues as expected and the results so obtained have revealed the general applicability of the principle involved.

IX. Studies in the Synthetic Application of Amidines

(M. V. SHIRSAT, K. GANAPATHI, R. C. SHAH)

Studies in the stereo chemistry of Amidines have been initiated with a view to throw some light on the hitherto little studied field in this series of compounds. The work is not yet completed but the results so far obtained indicate that a good contribution could be made in this direction.

X. Studies in the Synthetic Application of Amidines

(M. V. SHIRSAT, K. GANAPATHI, R. C. SHAH)

Synthetic applications of amidines for the purpose of converting carboxylic acids into the corresponding aldehydes are being studied further with a view to investigate whether or not the original method disclosed in the chemical literature could be successfully applied to aliphatic, hydro-aromatic and heterocyclic series. From the results so far obtained we feel justified in concluding that the method is atleast applicable to the aliphatic series in the first instance. Further work is under progress.

XI. Chemistry of Thiazole

(K. D. KULKARNI, K. GANAPATHI)

The chemistry of the thiazole ring is being studied further and attempts are being made to obtain some interesting derivatives which may come handy later on in the development of chemotherapeutical agents.

XII. The Influence of Tocopherol on the Production of Dietetic Necrosis of the Liver in Rats

(RADHAKRISHNA RAO, DATTA AND KRISHNAN).

In our previous experiments on the dietetic hepatic lesions in rats (reported before) acute massive necrosis of the liver or its sequels were not observed in animals fed on low protein diets (including those which received yeast as the main source of protein), as described by Himsworth and Glynn (1944). Even when similar diets have been used, there have been discrepancies between the results obtained in the different laboratories regarding the production of dietetic necrosis of the liver in animals. Himsworth claimed to have produced massive necrosis of the liver in 100 per cent. of the animals in his laboratory while others have succeeded in producing the lesion only occasionally. Radhakrishna Rao (Nature, *161*, 1948) enumerated the different factors which might be responsible for these discrepancies. In the meantime, experiments of Schwartz (1944) and Gyorgy (1947) seem to have indicated the influence of Tocopherol on the incidence of the liver necrosis in animals. To throw further light on this subject, the following experiments have been undertaken and are in progress.

(a) *The effect of pre-experimental diet on the development of massive hepatic necrosis in rats.*—It is considered likely that the stock diets may contain considerable amounts of tocopherol which ultimately interferes with the process of production of massive necrosis of the liver of the animals fed on yeast protein diet. This point is worth the attention for the success of the experiments on dietetic hepatic necrosis in animals. Accordingly, 36 young rats from 5 litters, with initial weights varying between 31 to 49 gms. on weaning, were distributed into two different groups with respect to weight, sex and litter mates. The rats were placed for 120 days on a diet calculated to be as low as possible in tocopherol. The diet was made up of alcohol extracted casein, Lard (as dietary fat), salt mixture, starch, sugar and supplement of 0.5 g. of dried brewer yeast (alcohol extracted). The diet of one group of rats, was however supplemented with tocopherol acetate. After 120 days, both the groups of rats were changed to yeast protein diet and no supplement of tocopherol was given to any group of rats. It is now nearly 150 days that the animals are getting the yeast protein diet. The records of daily food intake and body weights are kept. A few of the animals have been killed and the histological examination of the liver and the chemical analysis of the Glutathione content of the liver, kidney and muscle are in progress.

(b) *The prevention of the necrosis of the liver of rats by tocopherol.*—Two groups of rats have been placed on the yeast protein diet, similar in composition to that which Himsworth had found to be most effective in producing massive hepatic necrosis. One group of rats

(c) *Comparative effect of Lard and Hydrogenated vegetable fat on the production of massive hepatic necrosis in rats.*—All our diets used previously for animal experiments contained Hydrogenated vegetable fat as the fat moiety. Vegetable fats are fairly rich in tocopherol, while lard is devoid of it. This experiment has, therefore, been planned to study the relationship between the incidence of liver necrosis and the type of dietary fat.

XIII. Total Phospholipoid, Choline—containing Phospholipoid and Non-choline Phospholipoid in the Serum of Normal Human Subjects and in Patients with Cirrhosis of Liver

(RADHAKRISHNA RAO AND DATTA)

Total phospholipoid, choline—containing phospholipoid and Cephalin content of the serum of patients with cirrhosis of liver has been studied and compared with those of normal human subjects. In 11 normal human sera, lipid phosphorous was found, on an average 5.87 mg/100 c.c. serum and total phospholipoid was on an average 146.68 mg. per 100 c.c. serum. These values determined in normal persons are somewhat below the average normal standards given in literature for normal persons of the western countries. It was found that serum phospholipoids are predominantly choline containing type in normal persons. Non-choline phospholipoid (Cephalin) forms only a small fraction of the total phospholipoid present in the serum. These observations are in agreement with those of Entenman *et al* (1944). In cases of cirrhosis of liver with long-standing ascites, a marked reduction of the total phospholipoids, and particularly choline containing phospholipoids, has been found to be an important feature. Cephalin content of the serum, on the other hand, shows some increase over the normal values. It was found that with treatment and with partial clinical improvement, there was decided increase in the choline containing phospholipoids.

XIV. Cutaneous Manifestations in Experimental Vitamin A Deficiency

(RADHAKRISHNA RAO AND MISS KOLAH)

Young albino rats thirty days old with an average weight of 38 grammes, inbred in the Haffkine Institute were selected for the experiment. They were divided into four groups of six rats each.

Vitamin A—free basal diet was fed to all the animals. The diets of the first three groups were supplemented by 50, 5 and 1 I.U of vitamin A respectively while the fourth group received no vitamin A at all.

The duration of the experiment was about three months. The following symptoms were exhibited by the rats, especially those in groups III and IV—loss of weight, xerophthalmia, humped posture, nervousness and thin hair with a tendency towards epilation.

When the animals exhibited marked symptoms they were sacrificed by ether anaesthesia along with the corresponding ones in each group.

Skin pieces from various regions of the body were selected for detailed histo-pathological study. The tissues were fixed in 10 per cent. formal saline and blocks prepared according to the alcohol chloroform-paraffin technique. The sections were stained by Haemotoxylin-eosin, Van Gieson and Orcein Stains.

Histological examination of the tissues revealed the following changes :

Group IV.—Excessive hyperkeratosis of the stratum corneum, dilation and plugging of the hair follicles with keratinous material, atrophy of the sebaceous glands, slight monocytic infiltration of the corium especially in the perifollicular regions and total loss of subcutaneous and deeper fatty tissues.

Group III.—Hyperkeratosis of stratum corneum, dilation and plugging of the hair follicles with Keratinous material, slight atrophy of the sebaceous glands (only in some cases) and general loss of fatty, tissues.

Group II.—Only slight hyperkeratosis and dilation of the hair follicles could be detected in a few rats of this group. A good amount of fatty tissue was present in most of the rats of this group.

Group I.—(Control)—None of the animals in this group showed the presence of any of the pathological changes in the skin or subcutaneous tissues described above.

XV. Protein, fat and Phosphorus Content of Milk

(GOVIND)

Nitrogen estimations were done by the Microkjeldahl method using saturated boric acid for the collection of distilled ammonia. Protein values were calculated by multiplying the nitrogen value by 6.38. Babcock's centrifugal method was used in the estimation of fat. Phosphorus was estimated in the whole milk after digestion with 20 percent HCl, as well as in the filtrate after treatment of the milk with 50 per cent. trichloroacetic acid, according to the method of Fiske and Subbarow. The analysis data are presented below :

	Buffalo milk.	Cow's milk.	Human milk.
Number of samples analysed	30	10	20
Total proteins g/100 ml.	3.07-4.27	2.07-2.98	1.51-2.77
Fat g/100 ml.	5.90-7.60	2.90-8.60	2.60-4.00
Phosphorus total mg/100 ml.	25.0-40.0	12.5-23.8	4.80-10.60
Phosphorus in nonprotein nitrogen			

The human milk samples were obtained from the Nowroji Wadia Maternity Hospital, Bombay, through the kind courtesy of Dr. Patharkar, M.S. These samples were taken 5-15 days after delivery, as patients rarely stayed in the hospital for a longer period.

XVI. Choline Content of Milk

(GOVIND AND RADHAKRISHNA RAO)

The eniodide method of Ramon (1930) was used in the estimation of Choline.

Free choline was estimated in the filtrate after treatment of milk with 50 percent trichloroacetic acid and the choline in the combined form. was estimated after hydrolysis with barium hydroxide of the fat obtained by Soxhlet extraction of the airdried precipitate with methyl alcohol. The values are given below :

	Buffalo milk.	Cow's milk.	Human milk.
Number of samples analysed ..	30	10	20
Free choline mg/100 ml. ..	5.0-19.9	2.16-6.15	1.8-5.64
Choline in the fat portion mg/100 ml. ..	5.79-30.3	9.3-20.1	8.1-23.4

XVII. Methionine and Cystine in Milk

(GOVIND)

33 ml. of conc. Hcl. (sp. gr. 1.19) was added to 25 ml. of milk so that the whole solution contained 20 per cent. hydrochloric acid, and it was digested over a sandbath at 120° C for 10 hours. Methionine and Cystine were estimated from 10 c.c. portions of the digest, the former by the iodimetric method of Lavine (1942, 1943) and the latter by the Phospho-tungstic acid method of Folin and Merenzi (1929). The results are given below :

	Buffalo milk.	Cow's milk.	Human milk.
Number of samples analysed ..	30	10	20
Total Cystine mg/100 ml. ..	48-103	33-73	59-110
Methionine mg/100 ml. ..	76-233	70-185	39-81

XVIII. Studies on Blood Proteins in Health and Disease, and the Evaluation of Different Methods for Estimation of Blood Proteins in the Assessment of State of Nutrition in Population Groups

(MISS TARA R. KUNDAJI)

A comprehensive study has been undertaken to evaluate the various methods for the estimation of Blood proteins. Samples of blood collected from normal donors and those from diseased patients are analysed by the following methods :

1. Phillips and Van Slyke Specific gravity method.
2. Folin and Wu Phenol-Colorimetric method.
3. Kingsby Biuret method.
4. Kjeldahl Standard method.

Based on these investigations, a simple method, which can be applied in the field in the estimation of blood proteins in large population groups, will be worked out and the various details of the technique standardised.

XIX. Nutritional Requirements of Indian Strains of Lactobacilli and their Application in the Microbiological Assay of Vitamins

(COLAH)

The nutritional requirements of 6 strains of locally isolated Lactobacilli are being studied with a view to finding out their suitability for the microbiological assay of vitamins. A modification of the basal medium of Dunn and his collaborators is used to study the behaviour of the selected strains and this is compared with that of the standard strains.

The effect of limiting the amount of one vitamin in the basal medium on the utilization of another vitamin and the suitability of the modified medium for the assay of vitamins in natural foodstuffs are being studied.

XX. Effect of Different Fats on Fatty Infiltration of the Liver

(DESHPANDE AND GOVIND)

Ghee, 'Cocogem' (refined cocoanut oil) Ground Nut oil and Lard, were used at a 30 per cent. level in the diet of experimental animals to find out their effect on the fat, protein, and phosphorus content of the liver, and also to study the phosphatase activity.

The composition of the diet used is as follows : Fat 30 per cent., salt

were divided into 4 groups. A record of their daily food intake, and weights twice a week was kept. It was found that though the food intake of the rats on the different fats was about the same, there was an appreciable decrease in the liver weight and liver fat of the rats fed on ground nut oil, as compared with the animals getting the other fats. Liver protein, on the other hand, did not show any marked difference between the different groups. Studies on the phosphatase activity of the liver and kidney are in progress.

XXI. Nutritive Value of Amaranth (*Amaranthus gangeticus*)

(DESHPANDE)

Amaranth, a commonly used leafy vegetable, is a good source of proteins, minerals, and vitamins. Sufficient information however, is not available about the nature of the proteins and the minerals present in this food-stuff. This investigation was therefore undertaken to study the different fractions in detail.

The edible portion of the plant, i.e. the leaves, together with their petioles, were removed from the main stem by hand and dried in the sun for 2 to 3 days. The dried materials was then finely powdered and used for the analysis.

The results of the analysis of the above dried samples are given below :—

	Per cent.			
Moisture	..	..	..	8.7
Ash	..	..	..	17.1
Protein (N $\times$ 6.25)		..	..	29.7
Fat	..		..	4.7
Carbohydrates (by difference)		..	..	38.8
Sand and silica	..	..	..	1.1
Calcium	..	..	..	3.1
Phosphorus	..	..	..	0.13
Iron	..	..	..	0.124
Magnesium	..	..	..	2.5

The carbohydrate fraction was further studied in detail to find out the nature of the sugars present. The data are given below :—

	Per cent.			
Reducing sugars	..	..	..	0.047
Cane sugar	..	..	..	1.4
Starch	..	..	..	0.04
Crude Fibre	..	..	..	7.5
Pentoses	..	..	..	6.0

Investigations on the protein, fat, and phosphorus fractions are in progress.

XXII. Chemotherapy of Malaria

(SIDDONS)

The enquiry on chemotherapy of malaria under the auspices of the Indian Research Fund Association was begun in April 1948. The objective of the enquiry is the synthesis of compounds having prophylactic value against *P. vivax*. The lines of work proposed were the synthesis of potential antimalarials and the testing of these compounds against *P. gallinaceum* in chicks.

1. *Testing of compounds*.—A strain of *P. gallinaceum* was received in October 1948, through the courtesy of Col. Jaswant Singh, Director, Malaria Institute of India. The strain has since been maintained in the Institute in fowls and chicks. The testing of compounds has, however, been seriously impeded by the extreme difficulty of getting chicks in sufficient numbers from any source. Poultry breeders will only supply English breeds at a prohibitive cost. The few chicks used mainly for maintaining the strain, were incubator-hatched from commercial eggs whose fertility rate is low. These chicks, of course, breed are about half the weight of breeds used in standard procedures abroad. Inocula of 8 million to 24 million parasitized red cells were tried and 16 million was found to be a suitable density for testing of suppressive activity. Chloroquine synthesised in the Haffkine Institute and two new quinoline compounds have been tested in dosage based on known quinine equivalents of active compounds of this class. Chloroquine was active at 3 mg./kg.

Recent work in the U.S.A. suggests that there is a relationship between drug activity in the infected mosquito and in the sporozoite induced infection in the vertebrate, thus providing a new approach in the search for prophylactic drugs. Accordingly, we have started testing compounds against *P. gallinaceum* in *Aedes aegypti*.—Proguanil synthesised in the Institute completely inhibited development of sporozoites at a concentration of 0.001 gm. per cent. This confirms Terzian's observations with Paludrine. Eighteen of the most soluble new quinoline and sulphone compounds prepared have been tested in solutions of maximum solubility for toxicity to the mosquitoes, and fifteen of these for activity in infected mosquitoes, either in full concentration if not toxic, or in tolerated concentrations obtained by serial dilution, if toxic. The problems now confronting us are to prepare soluble salts or to try and administer insoluble compounds in artificial blood-meals by means of certain membrane-feeding techniques. Locally prepared gold beater skin and chick skin were tried without much success. A sample of gold beater skin was obtained from the U.K. and this is giving good results. Attempts were then made to administer drugs as solutions or suspensions in fowl plasma. 0.01 per cent. Proguanil for three alternate days inhibited the sporogony cycle, but 0.001 per cent. was not effective.

and six quinolines have been tried in infected mosquitoes in concentrations of 0.02 to 0.04 per cent., the solubility range in plasma. It has to be determined whether toxic doses can be administered by this method. As plasma is required in large quantity for this work the effect of horse plasma, more readily available than fowl plasma, on the sporogony cycle of the parasite is being noted.

XXIII. Potency Values of Single Dose Canine Vaccine

(DOGRA)

A canine vaccine previously prepared in this Institute was found devoid of any antigenic properties. Attempts were made, therefore, to prepare another canine vaccine which could meet the requirements of a prophylactic vaccine. The work was started in 1948 and continued during the year under report. Up to date seven brews have been prepared and assayed in Haffkine Institute inbred white mice. With single dose immunization LD 50 protection of vaccine showed a range between 4,169 to 21,880. These figures are above the prescribed level.

Experiments with stray dogs showed the vaccine to induce a satisfactory protection against an intracranial test virus infection. Laboratory inbred dogs are being reared to repeat the above work which will be undertaken during the coming year.

XXIV. Keeping Qualities of Semple's Vaccine

(DOGRA)

The expiry period of Semple's vaccine has been fixed on a purely arbitrary basis. In 1948 work was started to subject random samples of Semple's Vaccine to repeated assays and to study the keeping qualities of the vaccine. Two brews were taken and each one divided into two portions and one stored in a refrigerator at 5–8°C and the other at room temperature in the laboratory. These have now been tested for 10 months each. The results are set out in Table I.

TABLE I.—LD 50 potency value of Semple's Vaccine stored at room temperature and in a refrigerator (5–8°C).

Period of storage	Brew No. 77/49. 2 weeks 2,512		Brew No. 140/49. 2 weeks 22,910	
	Room	Cold	Room	Cold
2 months	..	1,445	1,778	46,770
4 months	..	75	0	4,467
6 months	..			14,130
8 months	..			9,333
10 months	..			under test

It was observed that whereas both the brews of vaccine when 2 weeks old showed a low potency value they both improved on storage in the cold and both markedly deteriorated when stored at room temperature.

Two fresh samples were taken and stored in the cold and assayed more frequently to observe the process of "Maturation" of the vaccine in the early weeks. The results of these two and the two tested previously are shown in Table II.

TABLE II.—LD 50 potency value of Semple's vaccine stored in refrigerator (5–8°C).

Age of Vaccine	Brew No. 77/49	Brew No. 140/49	Brew No. 822/49	Brew No. 825/49
2 weeks	2,512	22,910	19,500	33,110
4 weeks	..	..	72,440	72,440
8 weeks	43,650	46,770	100,000	61,660

The work is being continued to determine the period for which the vaccine could be stored with safety.

List of Papers published from the Haffkine Institute during the Year

1. DELIWALA, C. V., GANAPATHI, K. & RAJAGOPALAN, S. (1949) Chemotherapy of Tuberculosis. *Curr. Sci.* 18, pp. 233-239
2. DELIWALA, C. V., GANAPATHI, K. & RAJAGOPALAN, S.—Chemotherapy of Tuberculosis I. Synthesis of Possible Lipophilic Chemotherapeutics of the Sulphonamide and Sulphone Series derived from Fatty Acids including those of the Chaulmogr Group. In Press.
3. DELIWALA, C. V., & RAJAGOPALAN, S.—Chemotherapy of Tuberculosis II. Synthesis of N⁴-Acyl-5-Alkylsulphathiazolones and 5:5.—Alkylene bis-N⁴-Acyl Sulphathiazolones. In Press.
4. DELIWALA, C. V., & RAJAGOPALAN, S.—Chemotherapy of Tuberculosis III. Preparation of 5:5 Dichlorosalicyl and Related Heterocyclic Derivatives from Dichlorosalicyl and Related α -Diketones. In Press.
5. DELIWALA, C. V., & RAJAGOPALAN, S.—Chemotherapy of Tuberculosis IV. Synthesis of Dihydro- and Tetrahydro-2,4-Dichloro-5-Alkyl-1,3,5-Triazines. In Press.

6. DELIWALA, C. V., & RAJAGOPALAN, S.—Chemotherapy of Tuberculosis V. Synthesis of Some N^4 -Acyl- N^1 -Aryl Sulphanilhydroxamides. In Press.
7. DOGRA, J. R. (1949). A Single Dose Prophylactic Vaccination of Dogs against Rabies. *Ind. Vet. J.* 26, pp. 165-173.
8. NIMBKAR, Y. S., & LAHIRI, D. C. Inactivation of Cobra Venom with Soap Solution. In Press.
9. RAO, M. V. RADHAKRISHNA, DATTA, N. C. & KRISHNAN, L. S. (1949). Dietetic Hepatic Lesions and Protein Deficiency. *Cur. Sci.* 18, pp. 108-110.
10. RAO, M. V. RADHAKRISHNA, DATTA, N. C. & KRISHNAN, L. S.—Effect of Choline and Methionine on the experimentally produced Hepatic Lesions in Rats. In Press.
11. SADASIWAN, V.—Effect of Supplementary Zink on the Fat Content of Livers and Development of the Bones of Rats. In Press.
12. SHARIF, M. (1949). Effects of Constant Temperature and Humidity on the Development of the Larvae and the Pupae of the Three Indian Species of *Xenopsylla* (Insecta : Siphonaptera) *Physiol. Trans. Roy. Soc. Lond. Ser. B.* 233, pp. 581-635.
13. SOKHEY, S. S. & HABBU, M. K.—Aureomycin and Chloromycetin in the Treatment of Experimental Plague. In Press.
14. SOMAN, D. W. & NAIL, S. K.—A Study of the Bacterial Types of C. Diphtheria in Bombay. In Press.

P. M. WAGLE,

Parel, Bombay, 2nd April 1950.

Director, Haffkine Institute.

