



REPORT
OF
THE HAFFKINE INSTITUTE

For the Years 1944-1946

BY

Major-General Sir SAHIB SINGH SOKHEY, Kt., I.M.S.

Director, Haffkine Institute, Bombay.

BOMBAY

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEARS
1944-1946

PART I

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I. Staff.

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Department of Nutrition and Experimental Pathology

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Department of Clinical Pathology

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Department of Virus Diseases including Rabies

- Dr. R. Sanjiva Rao, M.B.B.S. (Madras), D.T.M. (Liverpool), B.M.S. (I). (*Assistant Director* from 24th May, 1946).
Dr. S. R. Savoor, M.B.B.S., B.M.S. (I). (*Assistant Director* up to 14th May, 1946).
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Blood Bank Department

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Mr. C. J. Karkhanis, B.Sc.

Scholars

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Miss B. M. Braganca, M.Sc. (Up to 30th June, 1946).

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Mr. H. B. Neogi, G.D.Art. (Bom.).

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Mr. Bakshamia Ramjumia	... Engineer.
Mr. S. R. Kulkarni	... Packing.

Junior staff, subordinates and inferior servants number 456.

II. General Review

The three years under review proved to be the most crowded years in the history of the Institute, both from the point of view of production of essential biological products needed by the country and of researches of great value. The Department of Vaccines had to be entirely re-equipped to meet unprecedented demands for the vaccines, which rose to 22 million cubic centimetres in 1945. The organization and equipping of the departments of Antitoxins and Sera and Chemotherapy were continued and successfully completed. Two new sections were organized to meet the urgent need of the country; one for making vitamin tablets, and the other for the production of solutions for intravenous use, such as protein hydrolysate and glucose saline. The former section produced 40 million vitamin tablets last year. The Institute is now considered one of the best equipped and the most active laboratories of India for medical research and for the production of the most needed biological products.

Research work, among other things, has yielded an entirely new cholera vaccine with ten times as high a protective power as the vaccine in use before. This vaccine will make a great mark in our fight against cholera. A very much improved plague vaccine with a consistently high protective power and a very much reduced toxicity has also been developed. This new vaccine has now replaced the old one which produced serious local and general reactions. Research work also raises the hope that in streptomycin we have an almost ideal remedy for the treatment of plague. Studies have led to the development of planned methods for the control of bubonic plague. There is also a possibility that the dreaded development of rash and swelling of joints resulting a week or so after the use of sera can be mastered. A new antivenene, which is effective against all the four common poisonous snakes of India—Cobra, Krait, Russell's Viper and Phoorse—has been developed, and is

issued in a dried form. In this form, it does not require to be stored in refrigerators; thus it can be kept and used even at small rural dispensaries. New methods for the manufacture of sulphadiazine drugs have been worked out, and methods for synthesizing antimalarial drugs are being studied.

The increase in the work of the Institute can be well gauged by the fact that in three years, its annual budget has increased from Rs. 7½ lakhs to approximately Rs. 18 lakhs, including the grant for vitamin tablets. The Institute has remained self-supporting ever since 1933, in that it has, by the sale of its products, recovered the total annual cost. The Institute has been able to achieve this by taking up more and more activities of practical value to the country. Though, it is primarily a medical research institute, it has taken a practical view of research as an intelligent method of solving problems. It is so organized that the officers in charge of the various departments are provided with adequate staff and leisure to conduct researches in addition to their routine production and analytical duties. The Institute has been fortunate in having secured the services of an excellent group of research workers. All this has been possible because the members of the senior staff are selected primarily on the basis of their original contribution to knowledge. During these three years, the workers of the Institute have contributed 42 original papers of considerable scientific value to the journals in this country and abroad, and equal number of them is in course of active preparation.

The demand for plague vaccine rose from the previous average of 4 million cubic centimetres a year to 15½ million cubic centimetres. Similarly, the demand for cholera vaccine rose to 6½ million cubic centimetres in 1945, while it was as little as ½ million cubic centimetres in 1940 and previous years. The maximum production capacity of the department was only 8 million cubic centimetres a year for the two vaccines. This necessitated re-equipping the whole department to increase its production capacity to 30 million cubic centimetres a year. Advantage was taken of this occasion to acquire the most modern equipment for vaccine manufacture.

The organisation of the Department of Antitoxins and Sera, which was begun in 1942, was continued and completed. The department now has excellent stables for 130 horses with 6 beautiful paddocks for exercising them. The Institute is grateful to Major C. C. Gulliland, Commandant of the Governor's Body Guard and Secretary, Royal Western India Turf Club, who took infinite trouble and personal interest in making the paddocks. This department has also been provided with 4 air-conditioned rooms complete with arrangements for sterilizing air for processing the sera. Thus this new department has now all the facilities for producing antitoxins and sera of the highest quality. The production of highly purified antitoxins, sera and toxoids by a national medical research laboratory of the standing of Haffkine Institute has met with a country-wide appreciation. This has encouraged the Institute to do more. The Institute now lyophilizes its purified antitoxins and sera to meet the special conditions prevailing in the country. Antitoxins and sera in liquid state, even when stored in efficient refrigerators, lose their potency in about two years. They suffer some deterioration even in transit over long distances in the country in hot weather. Prevailing atmospheric temperatures in the country are very high and refrigerators scarce. The result has been that small hospitals and dispensaries have had to do without these valuable therapeutic agents. The technique of lyophilizing, i. e. preserving labile biological products by drying them from the frozen state under high vacuum, has placed in our hands a very valuable weapon to meet these handicaps. When a liquid serum is frozen rapidly and dehydrated from the frozen state under high vacuum, a porous solid occupying essentially the same volume as the liquid from which it was prepared, is formed. Its content of antibodies suffers no detectable

loss in processing. The dried material can be stored at room temperature for very long periods without any loss of potency. Further, the porous dried product, on addition of distilled water, redissolves with remarkable ease and completeness. Therapeutic sera of the highest quality are now available to hospitals at $\frac{1}{2}$ to $\frac{1}{3}$ the price of imported sera.

The Department of Chemotherapy has been equipped with modern pilot plant, glass-lined and stainless steel kettles and other accessory equipment. With this equipment, a number of organic unit processes can be carried out at the Institute. The manufacture of sulphathiazole on a pilot-plant scale is in progress. This unit is imparting very valuable experience to the workers in large-scale plant operations, which should prove of great value if and when large-scale manufacture is taken up.

The Department of Entomology has proved most helpful in advising the departments of Public Health of the country in the newer anti-plague control measures. The recent war has brought to light some insecticides and rodenticides of great practical importance. Active researches are being carried on to determine the pulicidal value of DDT and 666 and rodenticidal value of "Antu" for Indian rats.

The Department of Nutrition has rendered great service to the Food Department of the Government of Bombay. All nutritional questions were referred to the department for advice, and the department carried out hundreds of tests and analyses of food-stuffs.

The most significant part of the activity of the Department of Clinical Pathology is that the diagnostic work in the case of infectious diseases, such as enteric fever, dysentery, diphtheria, typhus, etc., is carried out entirely free of charge for private practitioners. In addition, the charges for the various diagnostic determinations have been reduced to bring them within the reach of the members of the lower wage-earning groups of the city. Both these changes have proved of great value. The number of the samples examined by the department has risen from the neighbourhood of 8,000 to 18,000 a year.

In 1945, when the Province was faced with the risk of a possible famine, Government decided to make available vitamin tablets for scarcity areas. The only offer Government received for these vitamin tablets was from a commercial house at Rs. 60 per thousand. The Institute, however, took up the study of the problem and developed methods of extracting Vitamin A from shark-liver oil, and made arrangements to import the other vitamins from abroad and experimented with tablet making. These experiments were highly successful. In the middle of 1946, the Institute took up the manufacture of vitamin tablets. By the end of that year, the Institute had produced 36 million tablets of vitamin A and D and 4 million tablets containing ascorbic acid, thiamine chloride, nicotinic acid and riboflavin. The cost of production was Rs. 8 per thousand for A and D tablets and Rs. 10 per thousand for multivitamin tablets. At this low cost of production Government was enabled to do greater service to the Province than would have been possible otherwise.

III. Department of Vaccines

During the years under review, unprecedented demands for plague and cholera vaccines were made on this department. For the last two years, they amounted to more than 15 million cubic centimetres a year for plague vaccine, and to about 5 million cubic centimetres for cholera vaccine. The maximum production capacity of the department was only about 8 million cubic centimetres per annum. This necessitated the installation of lot of new equipment without stopping production even for a short while.

The demands came from all over India, which is a definite indication of the popularity of these vaccines with the public and a greater vigilance on the part of the public health authorities. It is imperative, however, that these prophylactic inoculations should be carried out in a more systematic manner than at present. Far too often they are started when epidemics have already broken out. The vaccines will serve a better purpose if epidemics are anticipated on epidemiological grounds and inoculations carried out well before the actual outbreaks.

A. PLAGUE VACCINE

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

Month	Quantities in cubic centimetres		
	1944	1945	1946
January	521,523	1,313,160	1,984,276
February	384,528	1,360,920	2,391,688
March	844,034	1,420,388	2,100,497
April	690,002	775,660	642,852
May	10,503	257,014	260,854
June	106,723	102,798	67,353
July	234,790	522,208	295,885
August	692,775	1,087,744	836,262
September	1,358,392	1,951,220	1,633,922
October	868,108	2,833,105	1,614,711
November	1,094,844	2,263,116	1,376,768
December	925,161	1,697,476	1,645,507
Total	7,731,383	15,584,809	14,850,575

The output 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	Cubic Centimetres
1897-1901	8,601,123
1902-1906	10,366,611
1907-1911	10,752,786
1912-1916	13,702,098
1917-1921	20,003,682
1922-1926	21,607,908
1927-1931	17,787,729
1932-1936	19,909,176
1937-1941	20,078,811
1942-1946	44,528,208
Total	187,338,132

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

				Quantities in cubic centimetres		
				1944	1945	1946
BRITISH INDIA						
Bombay Province	..	..	..	2,525,498	7,437,428	4,319,054
Madras Province	..	..	..	837,524	1,324,115	1,678,485
Bengal, Bihar and Assam	..	..	..	163,877	562,660	2,481,650
United Provinces of Agra and Oudh	..	..	..	1,124,842	1,225,544	3,148,941
Punjab	..	..	..	225,850	554,646	338,390
North-West Frontier Province	..	..	..	26	1,270	1,081
Central Provinces and Berar	..	..	..	463,623	331,248	306,160
Delhi Province	..	..	..	8,480	2,200	2,282
Sind Province	..	..	..	1,170	1,600	1,360
			Total	5,350,890	11,440,711	12,277,403
INDIAN STATES						
Southern Maratha States	..	..	..	329,760	756,012	302,280
Hyderabad Deccan	..	..	..	1,037,440	1,998,204	1,552,078
Kashmir	..	..	..	—	5,000	100
Mysore	..	..	..	822,402	1,191,082	144,230
Patiala	..	..	..	5,480	40,200	100
Jaipur	..	..	..	1,920	360	1,404
Nabha	..	..	..	—	1,000	6,000
Jodhpur	..	..	..	800	2,000	—
Bikaner	..	..	..	—	1,000	—
Indore	..	..	..	—	890	80
Cochin	..	..	..	—	250	14,000
Gwalior	..	..	..	37,400	—	100
Benares	..	..	..	34,000	35,000	4,000
Travancore	..	..	..	13,340	12,940	21,520
Bhopal	..	..	..	18,363	71,380	8,000
Rewa	..	..	..	8,000	12,400	4,000
Rajputana	..	..	..	308	—	1,380
Panna	..	..	..	2,600	1,600	500
Baroda	..	..	..	1,880	480	1,100
			Total	2,313,693	4,129,798	2,060,872

					Quantities in cubic centimetres		
					1944	1945	1946
FOREIGN COUNTRIES							
Aden	...	...	...	...	66,800	10,000	—
Portuguese Government					—	100	400
Muskat	...	..	...	..	—	500	—
Bahrain	...	...	...	...	—	200	—
Sharjah	..	..	..	...	—	2,000	—
Kuwait	..	...	..	..	—	1,000	—
Bushire	..	...	...	...	—	500	—
Burma	..	...	..	..	—	—	312,300
Total ..					66,800	14,300	312,700
Grand Total ...					7,731,383	15,584,809	14,650,975

B. CHOLERA VACCINE

When in 1933, the Institute took up the manufacture of cholera vaccine on a large scale, to meet the needs of the Public Health Department of this province, the anticipated annual demand was half a million cubic centimetres. The demand, however, has been progressively increasing, so much so that during the three years under review, about 15 million cubic centimetres of the vaccine were issued.

During this period, an entirely new cholera vaccine has been developed. The vibrio is grown in a liquid medium—casein hydrolysate. The new vaccine, by animal tests, is about 10 times more potent than the previous agar-grown routine vaccine in use. This new vaccine should give much higher immunity in human beings and raise the value of this vaccine as a preventive measure. Further details of this vaccine are given in Part II of this report.

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

Month	Quantities in cubic centimetres		
	1944	1945	1946
January	629,464	152,277	124,582
February	329,768	107,671	20,748
March	608,227	567,872	174,836
April	401,150	656,104	481,083
May	97,495	962,224	1,001,124
June	163,536	1,125,614	572,004
July	226,624	1,023,624	428,027
August	287,466	1,455,610	163,813
September	1,035,435	393,930	76,506
October	210,969	101,241	62,665
November	217,909	77,723	476,603
December	146,899	15,398	3,034
Total	4,354,942	6,639,288	3,585,025

C. T. A. B. AND OTHER VACCINES

The quantities of T. A. B. and other vaccines despatched monthly are shown below. The decline in the demand for anti-meningococcal vaccine is to be expected with the introduction of more efficient therapeutic agents against the disease :—

Month	Quantities in cubic centimetres					
	T. A. B. Vaccine			Anti-meningococcal Vaccine		
	1944	1945	1946	1944	1945	1946
January	7,829	9,740	6,535	—	20	—
February	7,831	19,826	12,781	—	—	—
March	13,313	15,897	2,691	—	—	—
April	15,808	25,867	7,085	—	—	—
May	4,762	11,458	16,314	350	550	—
June	18,831	13,470½	76,441	—	315	—
July	60,551	19,553½	181,090	—	—	—
August	46,193	25,286	43,206	—	—	—
September	16,859	16,089	15,139	—	—	—
October	14,729	12,709	28,692	—	—	—
November	20,521	5,680	6,950	—	—	—
December	5,366	4,543	1,941	—	—	—
Total	232,593	180,119	398,875	350	885	—

IV. Department of Antitoxins and Sera

During these years, the stables and paddocks for housing 130 horses were completed and all the serum production rooms were air-conditioned. Now the department can safely boast of the finest stables and paddocks in the whole of India. The department has set standards of excellence, which should prove of value to any other institution which may take up the manufacture of antitoxins and sera in India.

A new polyvalent anti-snake-venom serum effective against the venom of all the four common poisonous snakes of India, i.e. Cobra (*Naja naja*), Russell's Viper (*Vipera russelli*), Common Krait (*Bungarus coeruleus*), and Phooras (*Echis carinatus*), has been prepared, and is issued in the lyophilized form. As soon as the necessary equipment for lyophilizing large quantities of sera arrives, it is intended to issue all the different antitoxins and sera in the lyophilized form, in addition to supplying them in liquid form, as is done at present. This will meet the particular needs of this country, where the room temperatures are very high and refrigeration facilities are scarce, which cause deterioration of the therapeutic potency of the sera very rapidly.

Another very important advance in serotherapy has been made. An anti-allergic serum has been prepared, which it is expected, will eliminate or minimise the dreaded serum sickness and serum shock reactions which follow the use of antitoxins and sera. This anti-allergic serum is being incorporated with the different therapeutic sera issued by the Institute. Fuller details of this work are to be seen in Part II.

In November 1946 a case of epizootic lymphangitis was detected in the equine herd. This was followed by a few more cases in the herd under immunization. In consultation with the veterinary authorities steps were taken to check further spread of the disease. All the affected animals were destroyed. This untoward happening has made the creation of a permanent isolation ward for horses essential.

1. *Horses*.—Ninety horses were employed in 1944, 114 in 1945 and 106 in 1946 for the production of different antitoxins and sera. A number of preliminarily immunized horses, varying between 20 to 40, were always kept in reserve, which replaced those that were bled out or died. Two horses were kept for the supply of normal horse serum, which was required both for human use and for the preparation of media, etc., in the Institute. The horses were grouped as follows :—

For production of	Number of horses used		
	1944	1945	1946
1. Tetanus antitoxin	44	49	38
2. Diphtheria antitoxin	15	30	22
3. Anti-snake-venom serum	14	12	19
4. Welchii antitoxin	6	6	6
5. Septique antitoxin	1	2	2
6. Oedematiens antitoxin	2	3	3
7. Anti-plague serum	4	8	8
8. Anti-dysentery (Shiga) serum	4	4	4
9. Special anti-allergic serum	—	—	4
Total..	90	114	106

2. *Preparation of toxins and toxoids.*—Large quantities of different varieties of toxins and toxoids were prepared during the years under review. By far the greater part of these toxins and toxoids was used for hyper-immunization of horses. Small portions of tetanus and diphtheria toxoids were specially prepared for human use. A very small quantity of fully matured diphtheria toxin was used for the preparation of Schick toxin. The following table shows the quantity of each type of toxin prepared during the three years :—

Types of Toxins and Toxoids				Quantity in litres		
				1944	1945	1946
1. Tetanus	..	..	..	288	220	301
2. Diphtheria	..	..	..	124	173	102
3. Welchii	..	..	..	58	57	31
4. Septique	..	..	..	5	21	8
5. Oedematiens	..	..	..	12	26	31
6. Shiga	..	..	..	17	19	32
Total..				504	516	505

3. *Preparation of antitoxins and sera.*—The total volume of fully processed antitoxins and sera, which was made available for human use during these three years, amounted to 2,330,575 cubic centimetres. The heavy demand for anti-plague serum and anti-dysentery serum in 1945 was responsible for the greater manufacture of the natural serum. The following table gives the quantities of different kinds of antitoxins and sera prepared during the three years under report :—

Types of Antitoxins or Sera				Quantities in cubic centimetres		
				1944	1945	1946
1. Tetanus antitoxin	..	..	..	364,250	312,770	310,090
2. Diphtheria antitoxin	..	..	..	139,390	185,040	144,575
3. Gas gangrene antitoxin	..	..	..	45,970	53,210	109,130
4. Anti-snake-venom serum	..	..	..	24,820	85,040	106,660
5. Anti-dysentery serum (concentrated)	..	..	..	2,050	1,350	—
Total concentrated sera	..	..	..	576,480	637,410	670,455
6. Anti-dysentery serum (natural)	..	..	..	24,780	142,920	92,270
7. Anti-plague serum (natural)	..	..	..	44,930	80,170	47,810
8. Anti-allergic serum (natural)	..	..	..	—	—	13,350
Total natural sera	..	..	..	69,710	223,090	153,430

4. *Supply*.—The following quantities of antitoxins, sera and toxoids were issued during the three years under review :—

		Quantities supplied in cubic centimetres		
		1944	1945	1946
1. TETANUS ANTITOXIN				
(a)	Prophylactic—3,000 I. U. in 2 c.c. ..	158,270	259,478	307,918
(b)	Curative—20,000 I. U. in 10 c.c. ..	100,030	101,640	103,370
(c)	Curative—10,000 I. U. in 5 c.c. ..	—	1,165	2,815
(d)	Highly concentrated—20,000 I. U. in 4 c.c.	9,384	31,200	44,780
(e)	Diluted—1,000 I. U. per c.c. (bulk supply).	61,000	—	—
2. DIPHTHERIA ANTITOXIN				
(a)	Prophylactic—1,000 I. U. in 2 c.c. ..	12,568	3,260	1,970
(b)	Curative—10,000 I. U. in 10 c.c. ..	71,680	50,830	79,060
(c)	Curative—5,000 I. U. in 5 c.c. ..	—	620	2,325
(d)	Highly concentrated—10,000 I. U. in 5 c.c.	31,895	50,880	15,060
3. GAS GANGRENE ANTITOXIN				
(a)	3,000 I. U. Welchii antitoxin in 10 c.c. ..	30,440	38,480	63,580
(b)	3,000 I. U. Welchii, 1,500 I. U. Septique and 1,000 I. U. Oedematiens antitoxin in 4 c.c.	1,520	1,804	—
(c)	9,000 I. U. Welchii, 4,500 I. U. Septique and 9,000 I. U. Oedematiens antitoxin in 15 c.c.	14,505	11,205	14,415
4.	ANTI-PLAGUE SERUM in 20 c.c. ..	42,800	119,340	42,600
5.	LYOPHILIZED POLYVALENT ANTI-SNAKE-VENOM SERUM in 10 c.c.	—	45,200	44,370
6. ANTI-DYSENTERY SERUM (SHIGA)				
(a)	Natural, 4,000 I. U. in 20 c.c. ..	22,160	56,380	47,600
(b)	Concentrated, 4,000 I. U. in 5 c.c. ..	1,370	505	1,015
7. TETANUS TOXOID				
(a)	in 10 c.c.	3,600	36,440	18,970
(b)	in 5 c.c.	4,855	1,595	1,125
(c)	in 1 c.c.	462	141	551
8. DIPHTHERIA TOXOID ALUM PRECIPITATED				
(a)	in 5 c.c.	8,750	350	1,005
(b)	in 1 c.c.	706	309	308
9. LYOPHILIZED SCHICK TOXIN				
	Sufficient for 25 tests	—	15	10
10.	NORMAL HORSE SERUM in 10 c.c. ..	5,050	3,150	4,960

5. *Training*.—Dr. Liu Chun Hsiang worked on standardization of antitoxins and sera, Dr. Chau Chiu Shui on routine hyper-immunization of horses for the production of antitoxins and sera, and Mr. T. Velaudapillai on standardization of therapeutic sera. Dr. H. Mirdamadi worked on *in vitro* methods for standardization of therapeutic antitoxins, particularly diphtheria antitoxins, and prepared two papers on flocculation tests for diphtheria antitoxin. He was also explained the routine working of the department.

V. Department of Entomology

In addition to the usual routine work this department was engaged in researches mostly on fleas and their control. Ecological studies on the Indian rat-fleas, especially those dealing with the effects of constant temperature and humidity on the development of the larvae and the pupae of *Xenopsylla cheopis*, *X. astia* and *X. brasiliensis*, were prepared for publication. In order to ascertain the pulicidal effects of DDT and 666 series of experiments were conducted. They demonstrate a marked residual killing power of both these compounds, especially that of DDT, for fleas. They extend to us the hope that by use of the dust and other formulations of DDT and those of 666 also, it would be possible to exterminate fleas from plague-infected and other premises, including rodent burrows, and personal effects of their inmates. The results of these researches are summarized in Part II of this report, and on their basis suggestions for the use of DDT as anti-plague measure in its different formulations were made to the Director of Public Health for the Government of Bombay.

The department was also engaged in compilation, statistical calculation and tabulation of the flea and rodent data of different places in Sholapur, Dharwar and Belgaum districts surveyed by the members of the Plague Recrudescence Inquiry in Sholapur and Adjoining Districts. Plague-infected places in most of the districts of the Bombay Province during the years 1930-45 were plotted on skeleton maps with a view to study the progress of plague in the Province. On the basis of this study a statement about the forecast of plague epidemics during 1946 was furnished to the Director of Public Health of this Province.

During the three years many inquiries about fleas, other insects, ticks and rodents, and their control were attended to. The Department of Food Storage, New Delhi and Military authorities were supplied with information about Indian rodents and their control. Specimens of Indian rat-fleas and their developmental stages were sent to the Professor of Zoology, Calcutta University, and the Imperial Entomologist, Imperial Agricultural Research Institute, New Delhi. Exhibits and placards dealing with plague and anti-plague measures were given on loan for exhibition purposes. Professor C. Elton, Director, Bureau of Animal population, University of Oxford, was furnished with a sample of the "Elongated improved wire cage trap" along with its specifications.

Mr. C. C. Wu was given instructions in identification of fleas and in plague preventive measures. Major B. N. Khan, I.M.S. was explained for a week the use of DDT as a plague preventive measure. The 1944, 1945 and 1946 classes of sanitary inspectors and health visitors were given short course of instructions in plague and anti-plague measures. The staff of the Food Storage Department, New Delhi, was trained in identification of rodents and in the methods for their control.

The department was visited by three Americans and British experts on fleas and ticks engaged in typhus work in different theatres of war in Asia, and the procedure of plague survey work, adopted by the Plague Recrudescence Inquiry, was explained to them. The collection of ticks, fleas and lice sent by the following workers were identified :—

- (a) Major G. E. Davis, U. S. A. Army. (Ticks from Burma).
- (b) Dr. W. Dickinson, U. S. A. Army (Ticks from Agra).
- (c) Major S. L. Kalra and Captain M. G. P. Stoker, Base Typhus Research Team: (Ticks, fleas and lice from Poona, Naini-Tal District and Imphal).
- (d) Dr. H. N. Ray, Research Officer, Imperial Veterinary Research Institute, Mukteswar. (Ticks from Naini-Tal District).
- (e) Senior Entomologist, Department of Agriculture, Kuala Lumpur, Malaya. (Ticks from Malaya).

The department has greatly benefited, through the courtesy of the Secretary, Chemical Warfare Technical Committee, India, South East Asia Commands, Meerut, by the donation of "Reports of the Chemical Warfare Technical Committee", "Abstract Bulletins of the OSRD Insect Control Committee Co-ordination Centre" and "National Research Council, Abstract Bulletins of the Insect Control Committee Co-ordination Centre".

Samples of rat-traps were received for expert advice from different local bodies and private firms, and suggestions for the improvement of their construction were given to the rat-trap manufacturing firms. Advice was rendered to the Director of Public Health for the Government of Bombay and the Health Officers of local bodies in plague preventive measures.

A. EXAMINATION OF BOMBAY CITY RATS

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

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

TABLE I.—*Showing rats received at the Institute from the Bombay Municipality during 1944-1946*

Month	1944			1945			1946		
	Alive	Dead	Total	Alive	Dead	Total	Alive	Dead	Total
January ..	28,585	33,105	66,640	26,914	47,186	74,100	27,133	52,938	80,071
February ..	24,101	33,444	57,545	23,602	40,574	64,176	20,574	43,889	64,463
March ..	23,055	39,042	62,097	26,193	42,878	69,071	16,778	48,044	64,822
April ..	19,882	40,418	60,300	23,116	42,484	65,600	12,667	27,831	40,498
May ..	17,314	41,594	58,908	22,953	41,349	64,302	17,805	39,055	56,860
June ..	18,062	36,610	54,672	20,071	39,965	60,036	16,914	46,486	63,400
July ..	20,333	47,413	67,746	24,765	44,201	68,966	19,970	50,406	70,376
August ..	19,070	42,488	61,558	20,956	43,373	64,329	20,956	49,347	70,303
September ..	19,292	39,947	59,239	20,101	41,972	62,073	4,057	16,952	21,009
October ..	28,665	41,641	70,306	24,914	48,382	73,296	9,765	36,529	46,294
November ..	29,214	44,432	73,646	25,850	50,535	76,385	6,122	35,201	41,323
December ..	28,078	44,853	72,931	29,979	56,632	86,611	8,407	46,790	55,197
Total ..	275,601	480,987	765,588	289,414	539,531	828,945	181,148	493,468	674,616

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

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	5,203	23·0	4,345	21·7	4,001	23·4	3,220	21·5	3,719	24·5	3,446	21·8
<i>Rattus norvegicus</i> ..	5,439	24·0	4,162	20·7	3,823	22·3	3,171	21·2	3,329	21·9	3,684	23·4
<i>Gunomys kok</i> ..	9,982	44·1	9,486	47·3	7,760	45·4	7,132	47·7	6,944	45·7	7,148	45·3
<i>Bandicota malabarica</i> ..	440	1·9	436	2·2	245	1·4	308	2·1	118	0·8	181	1·1
<i>Mus dubius</i> ..	618	2·8	670	3·3	586	3·4	445	3·0	416	2·7	355	2·3
<i>Suncus caeruleus</i> ..	955	4·2	957	4·8	699	4·1	674	4·5	678	4·4	961	6·1
Unidentifiable ..	—	—	—	—	—	—	—	—	—	—	—	—
† Total ..	22,637	100·0	20,056	100·0	17,114	100·0	14,950	100·0	15,204	100·0	15,775	100·0

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

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

TABLE II.—*Showing dead rats examined for Plague during 1944-1946*

Month				Dead rats examined in 1944	Dead rats examined in 1945	Dead rats examined in 1946	Found plague infected in 1944-1946	
							<i>Rattus norvegicus</i>	<i>Rattus rattus rufescens</i>
January	..	..	..	24,475	33,668	34,317		
February	..	..	..	23,478	27,237	30,916		
March	..	..	..	24,192	27,848	27,651		
April	..	..	..	23,306	23,067	19,568		
May	..	..	..	24,907	26,612	32,918		
June	..	..	..	17,808	28,426	31,042	<i>NU</i>	<i>NU</i>
July	..	..	..	18,518	30,428	33,552		
August	..	..	..	15,061	26,186	28,816		
September	..	..	..	18,387	25,161	7,593		
October	..	..	..	24,632	27,002	21,297		
November	..	..	..	30,916	30,148	22,836		
December	..	..	..	27,027	34,295	28,469		
Total	..			272,707	345,078	318,974		

trapped alive by Municipal Corporation, Bombay during 1944*

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
3,717	22.7	3,662	25.2	3,383	23.1	4,347	20.0	4,675	19.3	4,087	19.4	47,805	21.9
3,652	22.3	3,151	21.6	3,250	22.2	5,149	23.6	5,240	21.6	4,169	19.7	48,219	22.1
7,220	44.1	6,187	42.5	6,621	45.2	10,813	49.6	12,539	51.7	10,840	51.4	102,672	47.0
242	1.5	260	1.8	146	1.0	177	0.8	379	1.6	587	2.8	3,519	1.1
422	2.6	428	2.9	391	2.6	451	2.1	475	2.0	477	2.3	5,734	3.0
1,037	6.3	872	6.0	807	5.5	858	3.9	771	3.2	891	4.3	10,160	4.7
83	0.5	—	—	59	0.4	—	—	147	0.6	33	0.1	322	0.2
16,373	100.0	14,560	100.0	14,657	100.0	21,795	100.0	24,226	100.0	21,084	100.0	218,451	100.0

often mutilated and even putrid,
not classified.

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

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	4,797	21.0	3,907	20.8	4,408	21.7	3,238	17.0	3,449	18.9	3,524	20.1
<i>Rattus norvegicus</i> ..	5,043	22.0	4,218	22.5	4,333	21.3	3,268	17.7	3,901	21.4	3,564	20.3
<i>Gunomys kok</i> ..	11,089	48.4	8,950	47.7	9,844	48.4	10,154	55.1	9,465	51.8	8,956	51.0
<i>Bandicota malabarica</i> ..	652	2.8	484	2.6	357	1.7	693	3.8	308	1.7	411	2.4
<i>Mus dubius</i> ..	577	2.5	505	2.7	485	2.4	474	2.6	449	2.4	442	2.5
<i>Suncus caeruleus</i> ..	721	3.3	672	3.6	930	4.5	599	3.2	690	3.8	650	3.7
Unidentifiable	3	0.0	16	0.1	—	—	—	—	—	—	—	—
†Total ..	22,882	100.0	18,752	100.0	20,357	100.0	18,426	100.0	18,262	100.0	17,547	100.0

* Rats included under this head though reported to have been caught alive

† These totals do not agree with the totals of live rats in Table I, because rats

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

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	4,764	22.8	4,113	23.8	2,443	21.0	2,147	19.7	3,253	18.3	2,659	18.1
<i>Rattus norvegicus</i> ..	4,566	21.8	3,923	22.7	2,965	26.2	2,830	26.0	4,453	25.0	3,477	23.5
<i>Gunomys kok</i> ..	9,975	47.7	7,979	46.1	5,205	46.2	5,369	49.2	9,057	50.9	—	52.3
<i>Bandicota malabarica</i> ..	330	1.6	213	1.2	63	0.5	16	0.2	123	0.7	187	1.3
<i>Mus dubius</i> ..	570	2.7	491	2.7	223	2.0	200	1.8	313	1.8	253	1.7
<i>Suncus caeruleus</i> ..	712	3.4	603	3.5	394	3.5	334	3.1	598	3.3	446	3.1
Unidentifiable	—	—	—	—	—	—	—	—	—	—	—	—
†Total ..	20,917	100.0	17,292	100.0	11,29	100.0	10,896	100.0	17,805	100.0	14,730	100.0

* Rats included under this head though reported to have been caught alive,

† These totals do not agree with the totals of live rats in Table I

of rats trapped alive by Municipal Corporation, Bombay during 1945*

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
4,479	22.5	3,464	22.8	3,708	24.5	4,801	23.1	3,699	20.4	4,518	20.0	47,992	21.0
4,415	22.2	3,432	22.5	3,630	23.9	4,606	22.2	3,940	21.7	4,837	21.4	49,187	21.0
9,190	46.3	7,215	47.4	6,669	44.0	9,809	47.2	9,055	49.9	11,763	52.3	112,159	49.2
306	1.5	151	1.0	103	0.7	110	0.5	251	1.4	302	1.3	4,128	1.8
553	2.8	357	2.3	429	2.8	538	2.6	408	2.2	466	2.0	5,683	2.5
924	4.7	611	4.0	626	4.1	924	4.4	805	4.4	696	3.0	8,848	3.9
—	—	—	—	—	—	—	—	—	—	—	—	19	0.0
19,867	100.0	15,230	100.0	15,165	100.0	20,788	100.0	18,158	100.0	22,582	100.0	228,016	100.0

were received at the Institute dead, often mutilated and even putrid.
received on Sundays and holidays were not classified.

rats trapped alive by Municipal Corporation, Bombay, during 1946*

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
2,970	17.8	2,479	16.2	495	17.0	1,221	16.8	1,116	21.4	1,087	17.5	28,750	19.6
3,984	23.9	3,497	22.8	629	21.6	1,570	21.6	1,212	23.3	1,509	25.8	34,710	23.7
8,536	51.1	8,374	54.5	1,579	54.2	3,867	53.2	2,487	47.8	3,071	49.4	73,207	49.9
161	0.9	197	1.3	27	0.9	111	1.5	69	1.3	124	2.0	1,621	1.1
386	2.3	355	2.3	59	2.0	169	2.3	109	2.1	105	1.7	3,203	2.2
662	4.0	447	2.9	125	4.3	333	4.6	215	4.1	225	3.6	5,094	3.5
—	—	—	—	—	—	—	—	—	—	—	—	—	—
16,699	100.0	15,349	100.0	2,914	100.0	7,271	100.0	5,208	100.0	6,211	100.0	146,585	100.0

were received at the Institute dead, often mutilated and even putrid.

because rats received on Sundays and holidays were not classified.

TABLE VI.—Showing the number and percentage of different species of rats

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	4,310	14.9	4,578	16.5	4,596	15.5	4,391	14.0	4,295	11.6	3,625	11.7
<i>Rattus norvegicus</i> ..	7,453	25.7	7,011	25.2	7,652	25.9	6,953	22.2	7,611	20.5	5,509	17.7
<i>Gunomys kok</i> ..	10,172	35.1	9,891	35.5	10,083	34.1	10,294	32.9	11,197	30.2	7,518	24.2
<i>Bandicota malabarica</i> ..	323	1.1	190	0.7	131	0.5	103	0.3	116	0.3	50	0.2
<i>Mus dubius</i> ..	2,005	6.9	1,618	5.8	1,515	5.1	1,346	4.3	1,434	3.9	940	3.0
<i>Suncus caeruleus</i> ..	2,415	8.3	2,866	8.5	2,510	8.5	2,747	8.8	2,812	7.6	2,971	9.6
Unidentifiable ..	212	0.7	190	0.7	215	0.7	219	0.7	254	0.7	171	0.5
Putrid ..	2,114	7.3	1,975	7.1	2,861	9.7	5,261	16.8	9,349	25.2	10,279	33.1
*Total ..	29,004	100.0	27,819	100.0	29,563	100.0	31,314	100.0	37,068	100.0	31,058	100.0

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

TABLE VII.—Showing the number and percentage of different species of rats

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ..	6,800	17.1	5,621	17.4	5,586	16.9	5,315	15.6	5,048	15.5	5,861	16.9
<i>Rattus norvegicus</i> ..	9,918	25.0	7,987	24.7	7,819	23.6	8,429	24.9	8,069	24.8	8,771	25.2
<i>Gunomys kok</i> ..	14,159	35.8	11,863	36.7	11,018	36.0	12,640	37.3	11,848	36.4	12,084	34.8
<i>Bandicota malabarica</i> ..	527	1.3	258	0.8	105	0.3	131	0.4	197	0.6	180	0.5
<i>Mus dubius</i> ..	2,089	5.3	1,478	4.6	1,417	4.3	1,432	4.2	1,202	3.7	1,263	3.6
<i>Suncus caeruleus</i> ..	2,790	7.0	2,220	6.9	2,521	7.6	2,660	7.8	2,237	6.9	2,334	6.9
Unidentifiable ..	—	—	30	0.1	—	—	91	0.3	248	0.8	267	0.8
Putrid ..	3,372	8.5	2,852	8.8	3,726	11.3	3,215	9.5	3,681	11.3	3,945	11.3
*Total ..	39,655	100.0	32,309	100.0	33,092	100.0	38,912	100.0	32,550	100.0	34,755	100.0

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

found dead in the City of Bombay during 1944

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
3,615	9.5	2,953	9.2	3,868	16.7	4,655	15.2	5,823	15.7	5,184	15.8	51,843	13.7
5,726	15.1	4,679	14.6	5,819	25.1	7,282	23.8	9,266	24.9	7,789	23.9	62,750	21.8
8,210	21.6	6,459	20.1	8,086	34.8	11,006	36.0	13,421	36.2	11,759	36.1	118,091	31.3
48	0.1	21	0.1	263	1.1	179	0.6	261	0.7	269	0.8	1,954	0.5
683	1.8	768	2.4	1,141	4.9	1,347	4.4	1,863	5.0	1,905	5.8	16,568	4.3
2,554	6.7	2,043	6.4	2,197	9.5	3,315	10.8	3,277	8.8	2,794	8.6	32,004	8.4
238	0.6	181	0.6	152	0.7	163	0.5	282	0.8	171	0.5	2,443	0.6
16,929	44.6	14,951	46.6	1,682	7.2	2,654	8.7	2,948	7.9	2,751	8.5	73,764	19.4
38,001	100.0	32,055	100.0	23,209	100.0	30,604	100.0	37,141	100.0	32,572	100.0	370,467	100.0

rats received on Sundays and holidays were not classified.

found dead in the City of Bombay during 1945

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
6,117	17.0	4,979	16.1	5,514	17.4	5,479	13.4	5,571	15.0	6,543	15.4	66,434	16.2
9,377	26.1	8,047	26.1	7,343	23.2	7,431	18.2	9,383	25.8	11,118	26.3	103,691	24.4
13,893	38.6	11,822	38.5	11,143	35.1	12,482	30.5	13,482	37.1	15,956	37.8	153,296	36.1
144	0.4	138	0.4	76	0.2	105	0.3	240	0.7	315	0.7	2,416	0.6
866	2.4	1,200	3.9	1,080	3.4	1,517	3.7	1,275	3.5	1,144	2.7	15,968	3.8
1,910	5.3	1,605	5.2	1,888	5.9	2,170	5.3	1,986	5.5	2,321	5.5	26,692	6.2
31	0.1	—	—	—	—	—	—	197	0.6	219	0.6	1,083	0.3
3,617	10.1	3,018	9.8	4,684	14.8	11,677	28.6	4,272	11.8	4,685	11.0	52,694	12.4
85,955	100.0	30,809	100.0	31,733	100.0	40,861	100.0	36,406	100.0	42,251	100.0	424,268	100.0

rats received on Sundays and holidays were not classified.

TABLE VIII.—Showing the number and percentage of different species of rats

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i>	8,229	16.3	5,891	16.2	5,954	16.0	3,332	13.6	5,523	14.2	5,256	12.4
<i>Rattus norvegicus</i>	10,797	26.5	9,232	25.4	8,194	25.0	5,993	24.4	9,289	23.8	9,007	22.0
<i>Gunomys kok</i>	16,475	88.0	14,822	39.4	13,102	41.6	9,482	38.6	16,927	43.3	15,071	38.0
<i>Bandicota matabarica</i>	1,397	1.0	1,413	1.1	382	1.2	193	0.8	265	0.7	331	0.8
<i>Mus dubius</i>	1,419	3.6	1,057	2.9	859	2.7	568	2.3	914	2.3	777	1.9
<i>Suncus caeruleus</i>	2,398	5.9	1,721	4.7	1,388	4.4	964	4.0	1,557	4.0	1,262	3.8
Unidentifiable	—	—	—	—	—	—	—	—	—	—	—	—
Total	40,728	100.0	36,372	100.0	31,624	100.0	24,533	100.0	39,055	100.0	40,958	100.0

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

B. EXAMINATION OF RATS FROM THE

These rats were received along with those of the other wards of the Bombay Municipality, previous tables. A separate record of these rats, giving the number and percentage Commissioner. Tables IX—XI give summaries of these records.

TABLE IX.—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>	511	58.8	299	80.8	312	53.0	214	47.5	119	23.6	103	19.3
<i>Rattus norvegicus</i>	58	6.7	71	9.5	35	6.0	17	3.8	91	18.1	97	18.2
<i>Gunomys kok</i>	147	16.9	173	23.0	108	18.4	156	34.0	217	43.0	244	45.8
<i>Bandicota matabarica</i>	13	1.5	7	0.9	5	0.8	3	0.6	1	0.2	2	0.4
<i>Mus dubius</i>	86	9.9	151	20.1	93	15.8	39	8.6	16	3.2	12	2.2
<i>Suncus caeruleus</i>	54	6.2	50	6.7	35	6.0	22	4.9	60	11.9	75	14.1
Unidentifiable	—	—	—	—	—	—	—	—	—	—	—	—
Total	869	100.0	751	100.0	588	100.0	451	100.0	504	100.0	538	100.0

Rats received on Sundays and

found dead in the City of Bombay during 1946

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
6,099	16·8	4,026	12·7	1,236	9·1	3,577	13·4	4,120	14·1	5,064	14·6	56,007	14·1
9,880	23·4	9,272	25·3	2,058	15·2	5,789	21·6	6,281	21·4	7,685	22·1	98,477	23·6
16,400	36·6	13,793	37·7	3,913	28·8	10,829	40·4	11,452	39·1	14,309	41·1	155,735	39·3
252	0·6	166	0·4	118	0·9	222	0·8	184	0·6	231	0·7	3,154	0·8
921	2·1	959	2·6	268	2·0	880	3·3	790	2·7	1,180	3·4	10,601	2·7
1,402	3·3	1,570	4·3	432	3·2	1,118	4·2	1,104	3·8	1,290	3·7	16,201	4·1
7,254	17·2	6,228	17·0	5,580	40·8	4,880	16·3	5,345	18·3	5,026	14·4	61,332	15·4
42,208	100·0	36,614	100·0	13,555	100·0	26,790	100·0	29,285	100·0	34,785	100·0	386,507	100·0

rats received on Sundays and holidays were not classified.

BOMBAY PORT TRUST DOCKS

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

species of rats caught alive from the Bombay Port Trust area in 1944

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
836	49·1	341	56·7	215	34·8	223	36·1	304	39·4	246	43·8	3,222	42·7
64	9·3	52	8·7	73	11·8	109	17·6	106	13·7	50	8·9	823	10·9
190	27·7	127	21·1	163	26·4	200	32·4	187	17·7	141	25·3	2,008	26·5
4	0·6	4	0·7	2	0·3	1	0·1	3	0·4	5	0·9	50	0·7
14	2·0	11	1·8	20	3·2	11	1·8	15	1·9	17	3·0	485	6·3
65	9·5	66	11·0	86	13·9	74	12·0	66	8·5	68	12·2	721	9·6
12	1·8	—	—	59	9·6	—	—	142	18·4	33	5·9	246	3·3
685	100·0	601	100·0	618	100·0	618	100·0	773	100·0	559	100·0	7,550	100·0

holidays were not classified.

TABLE X.—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> ..	320	50.6	180	32.1	207	37.7	285	53.5	187	39.5	234	36.7
<i>Rattus norvegicus</i> ..	78	12.4	64	11.4	87	15.8	58	10.9	93	19.7	70	11.0
<i>Gunomys kok</i> ..	183	29.0	275	49.0	180	32.8	127	23.5	140	29.6	259	40.6
<i>Bandicota malabarica</i> ..	1	0.2	1	0.2	2	0.3	1	0.2	1	0.2	4	0.6
<i>Mus dubius</i> ..	28	4.1	12	2.1	36	6.7	41	7.7	27	5.7	42	6.6
<i>Suncus caeruleus</i> ..	23	3.7	29	5.2	36	6.7	21	3.9	25	5.3	29	4.5
Unidentifiable ..	—	—	—	—	—	—	—	—	—	—	—	—
Total ..	683	100.0	561	100.0	548	100.0	533	100.0	473	100.0	638	100.0

Rats received on Sundays and

TABLE XI.—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> ..	295	42.9	309	46.1	118	24.1	236	41.3	316	50.5	285	47.1
<i>Rattus norvegicus</i> ..	154	22.4	66	9.8	108	22.1	95	16.7	118	18.1	45	7.4
<i>Gunomys kok</i> ..	189	27.5	194	29.0	286	48.3	195	34.1	143	22.8	209	34.6
<i>Bandicota malabarica</i> ..	5	0.7	12	1.8	2	0.4	4	0.7	5	0.8	5	0.8
<i>Mus dubius</i> ..	11	1.7	33	4.9	9	1.8	10	1.8	15	2.4	32	5.3
<i>Suncus caeruleus</i> ..	38	4.8	56	8.4	16	3.3	31	5.4	34	5.4	29	4.8
Unidentifiable ..	—	—	—	—	—	—	—	—	—	—	—	—
Total ..	687	100.0	670	100.0	489	100.0	571	100.0	626	100.0	605	100.0

Rats received on Sundays and

species of rats caught alive from the Bombay Port Trust area in 1945

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
423	56.9	198	46.0	256	46.0	240	35.3	193	44.1	309	42.6	3,032	43.6
86	11.6	33	7.7	54	9.7	121	17.8	53	12.1	93	12.7	890	12.8
175	23.6	167	38.8	216	38.9	269	39.6	139	31.7	246	33.7	2,376	34.1
3	0.4	3	0.7	5	0.9	1	0.2	5	1.1	3	0.4	30	0.4
13	1.7	6	1.4	3	0.5	8	1.2	13	3.0	46	6.3	275	4.0
43	5.8	23	5.4	22	4.0	40	5.9	35	8.0	32	4.3	358	5.1
—	—	—	—	—	—	—	—	—	—	—	—	—	—
743	100.0	430	100.0	556	100.0	679	100.0	438	100.0	729	100.0	6,961	100.0

holidays were not classified.

species of rats caught alive from the Bombay Port Trust area in 1946

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
263	33.6	276	41.4	70	39.6	281	51.1	295	56.1	165	41.8	2,909	43.1
130	16.6	69	10.5	11	6.2	59	10.7	28	5.3	19	4.8	897	13.8
301	38.8	241	36.2	80	45.2	147	26.7	138	26.2	160	40.5	2,233	33.1
5	0.6	—	—	2	1.1	3	0.6	4	0.8	—	—	47	0.7
16	2.0	31	4.6	8	4.5	16	2.9	11	2.1	15	3.8	207	3.1
70	9.0	49	7.3	6	3.4	44	8.0	50	9.5	36	9.1	454	6.7
—	—	—	—	—	—	—	—	—	—	—	—	—	—
785	100.0	666	100.0	177	100.0	550	100.0	528	100.0	395	100.0	6,747	100.0

holidays were not classified.

C. EXAMINATION OF RATS FROM STEAMERS AND OTHER SOURCES

During the years 1944, 1945 and 1946, 777, 686 and 1,045 dead rats and 37, 485 and 103 smears from dead rats respectively were received from different towns of the Bombay Province. As the result of their examination 10, 37 and 14 rats were reported as being plague infected in 1944, 1945 and 1946 respectively from towns mentioned in Table XII.

TABLE XII.—*Showing the different localities in the Bombay Province from which plague-infected rats were received during 1944-1946*

Name of Town	Number of plague-infected rats		
	1944	1945	1946
Ahmednagar	—	32	—
Alibag	1	3	—
Athani	—	2	—
Bijapur	—	2	—
Chipalunh	—	7	—
Chalisgaon	—	8	—
Igatpuri	—	2	—
Islampur	—	1	1
Jamner	—	2	—
Karad	—	2	—
Khanapur	1	—	2
Khed	—	6	—
Mahabaleshwar	1	38	2
Mahad	—	8	—
Mangaon	—	3	—
Panchagani	—	6	—
Pen	—	1	—
Pandharpur	5	—	—
Poona	1	—	—
Ratnagiri	—	6	—
Roha Astami	—	—	4
Sangamner	—	8	—
Sakharwadi	1	—	—
Sirur	—	—	5
	10	137	14

Blood smears from two dead cats were received from the Veterinary Assistant Surgeon, Hirekerur, for examination in 1945, and they were found positive to plague.

During the years under review 1,388 killed rats from steamers were received from the Health Officer of the Port of Bombay, and of these 1,293 belonged to the species *Rattus rattus rufescens*, 3 to *Rattus norvegicus* and 92 to *Mus dubius*. None of them was found plague infected.

VI. Department of Clinical Pathology

A. BACTERIOLOGICAL SECTION

This section examined clinical material from the various hospitals in the city and mofussil places in the province. Private medical practitioners also contributed a good number of samples for diagnostic work. Free diagnostic aid is given to all practitioners in infectious diseases, such as diphtheria, dysentery, cerebro-spinal fever, enteric fever, typhus fever, cholera, malaria, etc. The following table gives an idea of some of the important examinations carried out during the three years under report :—

Nature of examination	1944		1945		1946	
	No. of samples examined	No. of positive samples	No. of samples examined	No. of positive samples	No. of samples examined	No. of positive samples
Wasserman test	9,606	1,514	9,180	1,238	7,253	955
Kahn test	2,954	694	2,457	753	2,829	552
Widal test	1,738	413	1,566	461	1,561	249
Agglutination test for typhus ..	1,747	68	1,566	67	1,570	142
Agglutination test for Brucellosis ..	23	1	1,566	5	1,479	3
Sputum and urine for T. B. by smear and animal inoculation ..	303	78	168	70	161	43
C. S. F. smears, cultures ..	62	16	21	10	47	10
Throat swabs for diphtheria ..	69	15	61	21	478	133
Stools examination for intestinal pathogens	976	353	1,448	299	805	178
Blood films for malaria	162	46	354	63	505	87
Friedman test for pregnancy including 24-hour rat test	153	56	163	62	160	70
Rideal-Walker test	29	—	31	—	34	—
Autogenous vaccines	50	—	74	—	56	—

A marked increase in the number of samples received for Wasserman and Kahn tests is mainly due to a large number of samples sent by the Blood Bank Department. Though the City of Bombay was free from cholera during 1944, yet two stool samples were found positive. In 1945 as many as 151 strains of *V. cholerae*, chiefly of the Ogawa type, were isolated from over 1,000 samples of stools from the cholera patients. In order to find out the incidence of Brucellosis in Bombay City, every sample sent for widal was tested for agglutination for Brucellosis, and during 1945 and 1946 only 8 were found positive out of 3,045 samples. In 1945 and 1946, 175 strains of *C. diphtheria* were typed, 167 of these belonged to the "mitis" type, and 7 to the "gravis" and 1 to the "intermediate" type.

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

Year			Cobra venom in g.	R. viper venom in g.	Krait venom in g.	Echis venom in g.
1944	--	..	42.39	9.6	5.5	5.6
1945	--	..	11.60	6.7	4.5	12.0
1946	--	..	21.20	54.5	5.6	12.2

In addition, the section carried on the work of preparation of reagents for diagnostic purposes. Following reagents were supplied during the years 1945 and 1946 :—

Reagents			1945	1946
1. Blood grouping sera A & B	--	..	1,092 c.c.	1,732 c.c.
2. High titre sera	--	..	250 c.c.	346 c.c.
3. Killed suspensions—Widal & Weil-Felix	--	..	21,040 c.c.	51,883 c.c.
4. Kahn antigen	--	--	60 c.c.	50 c.c.
5. Amboceptor	--	--	8 c.c.	9 c.c.
6. JSB stain	--	--	60 lbs.	2 lbs.

B. SECTION OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY

This section mainly examined samples of waters for their fitness for drinking and industrial purposes, samples of milk mostly sent by the Civil Supplies (Secretariat) for bacteriological and chemical grading and detection of adulteration, and samples of butter and ghee for adulteration. Besides, two samples of peptone were received from the Government Medical Stores for testing their fitness for bacteriological work, a few samples of Sardines from the Chemical Analyser to the Government of Bombay for their fitness for human consumption, and samples of insecticides for testing their toxicity against mosquitoes. The

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

Samples examined	Nature of examination	Number of samples examined		
		1944	1945	1946
Water	.. Fitness for drinking purposes ..	28	31	18
Water	.. Fitness for bathing and swimming purposes	15	8	3
Water	.. Fitness for industrial purposes ..	3	5	5
Milk	.. For total counts and presumptive <i>B. coli</i> counts	35	—	216
Milk	.. For adulteration	6	—	—
Milk (human)	.. For bacteriological examination ..	—	—	1
Milk (condensed)	For bacterial flora	1	—	—
Butter	.. For R. M. Value and adulteration ..	—	—	2
Ghee	.. For adulteration	1	—	—
Sardines	.. For fitness for human consumption ..	2	—	—
Peptones	.. For fitness for bacteriological work ..	2	—	—
Meat (dehydrated)	For bacterial counts	—	—	3
Insecticides	.. For toxicity against mosquitoes ..	2	—	—

VII. Department of Pharmacology

This Department was chiefly concerned with the examination of drugs on behalf of the military authorities. Although the department has been taken over by the Government of Bombay, the military authorities still continue to bear part of the cost of the department.

1. *Examination of drugs, chemicals and apparatus on behalf of the military authorities.*—This work was continued during the years under report on the same lines as was done in previous years. The Officer Commanding, Medical Stores Inspection Depot, sent samples which were chiefly British Pharmacopoeial drugs, including biological products, chemicals, anti-mosquito sprays and creams, and ampouled products for sterility tests. The number of samples examined was 1,962 in 1944, 1,344 in 1945 and 199 in 1946. Various methods were involved in examining these samples, the important ones being chemical examinations both qualitative and quantitative, pharmacognostic, bacteriological and biological methods. Animal experiments for detection, identifications and assays were also employed. Standardization and calibration of apparatus were also undertaken.

2. *Manufacture of Mydracaine*.—At the special request of the Director of Medical Services, General Head Quarters, New Delhi, the department had undertaken to supply mydracaine solution in ampoules to the army. Total supply made during the three years under report was 6,692 ampoules.

3. *Preparation of sulphanilamide-cod-liver-oil paste for civil use*.—The advantages of this paste in "Tropical Ulcer" are now well recognized, and the department has undertaken to supply the needs of the Bombay Province. Medical Officers in the province indent for this paste, and all their demands during the three-years were fully met. These were 392 lbs. in 1944, 97 lbs. in 1945 and 272 lbs. in 1946.

4. *Standardization of protein hydrolysate for intravenous use*.—The impending famine in the province necessitated the preparation of non-pyrogenic injectable protein hydrolysate. Both chemical and biological tests were employed. The product which passed these tests did not produce any untoward reaction when tried clinically. Casein hydrolysate was made available for issue to hospitals.

5. *Standardization of drugs sent by manufacturers*.—Manufacturers of biological products sent their products for assay to this department. They consisted chiefly of Tincture of Digitalis and Strophanthus, Adrenaline, Pituitrin, and Liquid Extract of Ergot. Samples of organic arsenicals were received for determining their toxicity. Several chemicals were also received for B. P. Tests including chemical assays.

6. *Miscellaneous work*.—The examination of drugs for their alleged curative action in snake-bites, identification of snake venoms in solutions suspected of containing them, identification and estimation of quinine in tablets and ampoules, examination of distilled water for presence of pyrogens, etc., were carried out. Water tanks and containers were received for examination to detect if lead was present, as they were to be used for transporting water.

7. *Research work*.—Pressure of routine work left little time to pursue research problems. However, some investigations on the action of ions on the central nervous system, standardization of protein hydrolysates, and the chemotherapy of intestinal diseases were carried out in the department. The work on the local treatment of infected experimental wounds was pursued further. The results of these investigations are given in Part II of this Report.

VIII. Department of Chemotherapy

During the years under review, the scope and activities of this department have expanded considerably. Investigations on the production of important essential drugs on a commercial scale, and researches in the chemotherapy of malaria and bacterial infections are the two main lines on which work is proceeding. A very valuable addition to this department has been made by the organization of a section for the production of vitamin A concentrates and vitamin tablets.

Investigations on the production of the important sulpha drugs, sulphathiazole and sulphamerazine, were continued. Eight patents have so far been taken out covering the processes of manufacture of these drugs and the intermediates. In the manufacture of sulphathiazole, a number of improvements in the processes have been effected particularly in the preparation of the intermediate, 2-aminothiazole. A well equipped, modern pilot plant laboratory with glass lined and stainless steel kettles and other accessory equipment has been fitted up. With

this equipment a number of organic unit processes can be carried out. The manufacture of sulphathiazole on a pilot plant scale is in progress. This unit is imparting very valuable experience to the workers in large scale plant operations, which will yield data for the manufacture of drugs on a large scale, and will help the design of suitable manufacturing units. The manufacture of the important anti-malarials are also being investigated. This research is being actively conducted. The results are of considerable interest.

The department supplied all the sulphathiazole required by the Blood Bank as a plasma preservative, and all the phenyl mercuric nitrate used as the preservative in the place of phenol in preparation of vaccines at the Institute. The antiseptic "pemon" was also produced to meet the needs of hospitals.

Researches on the chemotherapy of bacterial infections were continued. In the course of this work, more than two hundred new derivatives of the sulphanilamide and sulphone group were synthesized. A number of representative compounds were tested in experimental plague, streptococcal and pneumococcal infections in mice for their therapeutic properties. Though no compound of outstanding activity was discovered, very interesting data throwing light on the relationship between chemical constitution and chemotherapeutic action have been obtained. This knowledge is of great importance because it will help us to develop a rational basis for the design of chemotherapeutic agents. The results obtained in the course of this work are given in Part II of this Report.

Malaria being the problem of paramount importance to the country, researches on the chemotherapy of malaria have been instituted. The ultimate aim of this work is to find solution to the two important practical problems: discovery of drugs that would be at least as effective as the best ones we have got, but would be much easier and cheaper to produce in the country, and discovery of compounds that would produce a radical cure in benign tertian malaria which will destroy the exoerythrocytic forms of *Plasmodium vivax* and at the same time be free from toxicity. With this aim, syntheses of compounds of the quinoline, thiazole, guanidine and biguanidine types are in progress.

Compounds of the thiazole group offer promise as good therapeutic agents; but our knowledge about the chemistry of the thiazole was too meagre to help us in synthesizing compounds of particular groups and structures required. For this purpose, the chemistry of the thiazoles is being studied in great detail. Methods of synthesis of the 4- and also 5-aminothiazoles and their derivatives have been worked out. Great improvements have been made in the synthesis of the 2-halogenothiazoles which are very valuable intermediates in many synthetic operations and also for the preparation of sulphathiazole by an alternative route. The different methods of preparation of thiazole compounds unsubstituted in position 2 have been investigated to ascertain the best method of preparation of 4-methyl-5-hydroxyethylthiazole, an intermediate in the manufacture of the important vitamin, thiamine chloride.

When it was realised that penicillin as a very valuable chemotherapeutic agent has come to stay, attempts were made to study the production of this drug in the laboratory. A number of strains of *Penicillium notatum* were collected, and studies were made to find out the most suitable medium, the best method of growing the mould, and also the method of extracting penicillin from the culture fluid giving the highest yield. Because of the hostilities at that time, the information on the

above points were kept secret in U.K. and U.S.A., and so was not available at this end. Now we are in possession of all these details. Recently as a result of work done in U. S. A., strains of *Penicillium chrysogenum* have been produced by irradiation and mutation, which furnish very high yields of penicillin. Also the moulds have been found to produce at least four different types of penicillins depending upon the conditions of culture and the nature of the mould. A number of these new strains have been obtained, and these are being studied to ascertain the nature of the penicillins they produce. The estimation of the penicillin by the iodimetric titration method has been standardized. This is found to be very convenient, very rapid and much better than the usual biological methods for routine work. Methods are being tried to differentiate between the four penicillins by chemical methods and chromatography.

With a strain of *Actinomyces grieseus* obtained from Dr. Waksman, the production of streptomycin in surface cultures was investigated. A large number of different media and different methods of inoculation were tried. Though good growth of the mould was obtained, the yield of streptomycin was not as it should be. The strain was found to be a poor streptomycin producer.

VITAMIN SECTION

As a result of war and the introduction of rationing in the country, diseased conditions traceable to the deficiency of vitamin A began to appear. The Government planned to combat this by the administration of vitamin A to the vulnerable section of the population. Since shark liver oil possesses a smell which is not tolerated, it was proposed to obtain vitamin A concentrates free from the objectionable odour. This department was asked to investigate the preparation of vitamin A concentrate from shark liver oil. When the preliminary experiments were successful, a small staff was sanctioned for further large scale investigations. A good method of preparing vitamin A concentrate in high yields from shark liver oil was developed. To carry on the work on a larger scale, additional staff was sanctioned by the Government. Since the concentrates prepared are usually of potency of about 200,000 to 500,000 units of vitamin A per gram, the method of administration of about 2,000 units of this vitamin to the individuals presented a problem. Addition of this concentrate to milk was found to be unpracticable for many reasons, one of the important reasons being that a good portion of it is destroyed by the usual method of boiling practised in the household. The problem was solved by incorporating the vitamin into tablets with suitable packing and flavouring materials. Thus tablets are now being manufactured each containing 2,000 units of vitamin A and 200 units of vitamin D. These tablets are attractive, liked by children, and the vitamin in them do not undergo destruction. They are ideal for distribution on a mass scale.

The process of the manufacture of the vitamin A concentrate has been well standardized, and research is also going on to improve the product further. The concentrates prepared are attractive and have potencies of about 200,000 to 800,000 units of vitamin A per gram. Up to the end of 1946 alone, 1,296 gallons of shark liver oil were thus concentrated. Now the capacity of this section has been increased to handle about 500 gallons of the shark liver oil per month. With this set up, practically all the shark liver oil produced in the Bombay Presidency could be concentrated.

A very good tableting section on very modern lines has been set up, with two big rotary tableting machines, oscillating granulator, driers, mixers, etc. This section can produce about 4 to 8 million tablets per month in the normal course, and the capacity could be increased further if there is a need. Up to the end of 1946, this section has produced 36·1 million vitamin A and D tablets. Of these 22·5 millions were dispatched to the various places according to the directions of the Government. In addition, multivitamin tablets containing ascorbic acid, thiamin chloride, nicotinic acid and riboflavin have been prepared. So far 3·8 million of these tablets were prepared and 2·3 millions were dispatched to the various places. The cost of these tablets is fraction of the prevailing market prices in India. This department has thus made a very great saving to the Government. Researches are still going on to further cut down the price of the tablets.

The vulnerability of the population to the vitamin deficiency diseases is an important problem for us at present and it is bound to remain so for a long time to come. So there will be a great need for these vitamins in the country. This section will therefore be of very great value to the Government. The Government has therefore been requested to put this department on a permanent basis. If this done, more extensive work could be undertaken in this field.

IX. Department of Biochemistry

This department has continued as before to give useful aid to hospitals and medical practitioners by carrying out the various biochemical examinations necessary for the diagnosis of diseases. It gave assistance to other departments of the Institute in their research work.

1. *Nutritional work.*—The effect of fortification of wheat flour by calcium carbonate on two of its nutritionally important constituents, viz., vitamin B₁ and protein content has been studied.

2. *Respiration of plague bacillus.*—Using Warburg's manometric technique, the study of oxidation of different substrates by virulent and avirulent strains of plague bacillus has been completed.

3. *Examination of milk samples.*—To assist the Milk Distribution Scheme of the Government of Bombay, the routine examination of milk samples collected from the different distribution centres in Bombay and Suburbs, was carried out. Since the inception of the scheme the number of samples tested was 7,038 including 1,956 'Anand' samples.

4. *Routine examination.*—The number of routine examinations carried out during the years under review was 5,329 : blood 2,715 ; urine 654 ; cerebrospinal fluid 618 ; faeces 58 ; milk 184 ; special tests 335, such as 129 gastric analysis, 76 blood urea clearance, 91 Basal metabolic rate determination and 39 glucose tolerance tests ; other assays, such as 572 Vitamin A, 82 Vitamin B₁, 85 Vitamin C, 4 Riboflavin, 3 Nicotinic acid, 6 Vitamin D, 13 samples of wheat flour, soya beans and creta preparata. The routine examinations were mostly determinations of non-protein nitrogenous constituents such as urea, uric acid, sugar, cholesterol, serum proteins, calcium, haemoglobin, Van den Bergh reaction, blood sedimentation rate, etc.

X. Department of Nutrition and Experimental Pathology

A. NUTRITION SECTION

This section continued to render useful service to the Civil Supplies Department of the Government of Bombay. Various questions on human nutrition arising out of the rationing of food-stuffs in the province were referred to this Institute, and the section has given technical advice in matters of practical nutrition. The section undertook analytical work of nutritional type, e.g. analysis of food-stuffs for their proximate principles, estimations of vitamins, etc., both for government institutions and the public. It also carried out vitamin estimations in connection with the research and routine work which includes assay of vitamins in the vitamin tablets prepared at the Institute. All the analytical work was carried out in collaboration with the Department of Biochemistry of this Institute.

During the year 1944, experimental work in connection with the fortification of wheat flour with calcium carbonate was undertaken on behalf of the Finance Department (Supply) of the Government of Bombay. It was found that the addition of calcium carbonate to wheat flour in a proportion of 1 in 320 did not have any deteriorating effect on the protein and vitamin B₁ contents of the latter under ordinary conditions of storage for four weeks. Most of the Indian diets consumed by the poorer classes are deficient in calcium, and the fortification of wheat flour with calcium carbonate is an important step in improving them. For this purpose, the availability of calcium carbonate from local sources was investigated owing to the high cost of the imported material. Analysis of several samples of calcide showed that pure calcium carbonate is available in a natural form in India. It is stated that large quantities of this material are available, and it is very cheap.

At the request of the Central Government, an extensive investigation was carried out on roller-processed skimmed milk powder intended for human consumption.

During the year 1946, the Government of Bombay had taken up the problem of incorporating groundnut cake atta in ordinary food-stuffs. Experimental work in connection with the removal of oil from the groundnut cake was undertaken. It was found that double crushing of the cake had succeeded in reducing its oil content from 15 to about 9 per cent.

About the same time experiments were conducted in this section on reconstitution of separated milk powder. As skimmed milk powder has practically no fat, experiments to add fat in the form of deodorized coconut oil were carried out. For this the method recommended by the Ceylon Trade Commissioner for India, was followed. The following formula was used.

Water	...	...	...	...	...	100 gallons.
Skimmed milk powder	...	...	...	...	...	100 lbs.
Deodorized coconut oil	...	...	...	...	...	50 lbs.
Concentrated shark liver oil	...	...	...	...	...	1 oz.

The reconstituted milk thus prepared was quite palatable, the only drawback being a tendency for the fat to separate out when kept in the cold at 7°–10°C. (45°–50°F.).

Experiments were also conducted at the Institute to see whether *Mahua* flowers could be incorporated in ordinary food-stuffs and palatable preparations could be made therefrom. The results were on the whole disappointing. The flowers though sweetish in taste have a peculiar flavour, and they cannot be consumed in large quantities.

This section served as a centre for training in human nutrition. Towards the end of the year 1944, the Finance Department (Supply) appointed a Nutrition Officer mainly to undertake field work in nutrition and carry out rapid nutrition and diet surveys in the province. This officer was trained in the department for the work, and he is at present working in close co-operation with the section. Two Lady Dietetic Officers appointed by the Government of Bombay were also trained in the principles of human nutrition and dietetics. Similarly, a Medical Officer deputed by the Government of the Central Provinces and the Nutrition Officer, Public Health Department, Government of Punjab, were given training in food-deficiency diseases and laboratory technique as applied to nutrition work.

Under the auspices of the Indian Research Fund Association a research enquiry was commenced during the year 1944 to study the possible role of nutritional factors in the production of cirrhosis of the liver. Portal cirrhosis is a common disease in certain parts of India, mostly amongst the poorer people, and the aetiology of the condition is obscure. Experimental work in connection with this enquiry is in progress.

The department affords facilities for post-graduate research work. Miss Beatriz M. Braganca, M.Sc., who was appointed by the Dorabji Tata Trust as a research scholar, Bombay, commenced work in the section in 1945 on the problems connected with vitamin K. Very little attention has hitherto been paid by Indian workers to studies in vitamin K, and it is expected that the work which is being carried out in this section will add to our knowledge of this subject. A brief account of the researches carried out so far is given in part II of this Report.

The section maintained contact with the Food Department of the Central Government, Nutrition Research Laboratories, Coonoor, the Nutrition Advisory Committee of the Indian Research Fund Association, the All-India Nutrition Board and nutrition workers both in India and abroad.

B. PATHOLOGY SECTION

During the years under review, 3,187 pathological specimens, received from different hospitals in the province, were examined. They include brains from suspected rabid animals, neoplasms, gynaecological conditions, tuberculosis of different tissues and some rare and interesting pathological specimens. A rough classification of the morbid histological condition of the tissues received at the Institute during the three years and their numbers are given in Table I.

TABLE I.—*Showing the number of pathological tissues received and examined during the years 1944, 1945 and 1946*

	1944	1945	1946
I. NEOPLASMS			
<i>(a) Malignant tumours</i>			
(1) Carcinoma primary :—			
Squamous-cell carcinoma	9	18	18
Basal-cell carcinoma	1	—	4
Adenocarcinoma	7	6	5
Carcinoma simplex	2	6	3
Carcinoma embryonal	2	—	—
Carcinoma medullary	1	1	1
Carcinoma mucoid	—	—	2
Carcinoma scirrhus	—	6	11
Carcinoma alveolar	—	—	2
Carcinoma papillary	—	3	5
Carcinoma bile duct	—	—	1
Chorionepithelioma	—	1	2
Hypernephroma	1	—	—
Seminoma	—	1	1
(2) Carcinoma secondary	4	7	4
(3) Sarcoma :—			
Fibrosarcoma	4	3	8
Round-cell sarcoma	1	—	2
Neurogenic sarcoma	—	—	1
Osteogenic sarcoma	—	2	2
Spindle cell sarcoma	—	—	1
Melanotic sarcoma	—	1	—
(4) Malignant lymphoma :—			
Lymphosarcoma	1	2	2
Hodgkin's sarcoma	—	1	1
<i>(b) Non-malignant tumours</i>			
Adenoma	5	1	—
Cystadenoma	1	1	—

				1944	1945	1946
Papillary cystadenoma	...	...	...	—	2	—
Pseudomucinous cystadenoma	...	...	...	2	—	3
Fibroadenoma	...	...	...	2	8	—
Adenomyoma	...	...	...	2	5	—
Fibromyoma	...	...	...	14	18	13
Fibroma	...	...	...	9	5	2
Fibrolipoma	...	...	...	3	2	—
Neurofibroma	...	...	...	2	1	3
Colloid goitre	...	...	...	3	1	3
Lipoma	...	...	...	1	3	5
Osteochondroma	...	...	...	1	—	—
Cancellous osteoma	...	...	...	1	—	—
Papilloma	...	...	...	2	8	3
Polypus	...	...	...	17	28	28
Angioma capillary	...	...	...	—	3	8
Angioma cavernous	...	...	...	—	4	—
Lymphangioma	...	...	...	—	1	—
Myxoma	...	...	...	—	1	—
Pigmented nævus	...	...	...	—	2	—
Osteoclastoma	...	...	...	—	2	—
Melanoma	...	...	...	—	—	2
(c) <i>Mixed tumours</i>	...	...	...	1	1	—
(d) <i>Teratoma</i>	...	...	...	2	—	—
(e) <i>Endothelioma</i>	...	...	...	2	1	—
Rhinosporidiosis	...	...	...	3	—	—
Mycetoma	...	...	...	—	—	1

II. TUBERCULOSIS

Lymph-nodes	...	...	...	8	10	7
Breast	...	...	...	—	2	1
Female genital tract	...	...	...	12	17	10
Male genital tract	...	...	...	—	—	2

				1944	1945	1946
Peritoneum	...	...	...	1	—	—
Buttocks	...	...	...	1	—	—
Stomach	...	...	...	2	1	—
Anus	...	...	...	1	1	2
Foot	...	...	...	—	1	1
Forearm	...	...	...	—	1	—
Ileum	...	...	...	—	1	—
Leg	...	...	...	—	1	1
Thigh	...	...	...	—	—	1
Palm	...	...	...	—	—	1
Liver	...	...	...	—	—	1
Heart	...	...	...	—	—	1
Lung	...	...	...	—	—	1
Perineum	...	...	...	—	—	1
III. RARE GYNAECOLOGICAL CONDITIONS						
Cysts of the corpus luteum	...	...	...	6	6	8
Ectopic pregnancy	...	...	...	8	11	17
Dermoid cysts	...	...	...	—	3	2
IV. MISCELLANEOUS PATHOLOGICAL CONDITIONS (including tissues from experimental animals)						
	...	...	...	522	818	752
V. TISSUES FOUND TO BE NORMAL ON HISTOLOGICAL EXAMINATION						
	...	...	...	76	45	29
VI. TISSUES RECEIVED IN A STATE UNFIT FOR EXAMINATION						
	...	...	...	7	15	9
VII. BRAINS OF ANIMALS FOR EXAMINATION OF NEGRI BODIES						
	...	...	...	145	113	90
Total				895	1,208	1,084

The regional distribution of the malignant tumours is given in Tables II, III & IV. The increase in the number of specimens examined, as compared to previous years, is largely due to the activities of the Experimental Pathology Section where the problem of hepatic cirrhosis is being studied. This section continued to give assistance to other research enquiries conducted at the Institute.

Some of the interesting specimens received during this period are mentioned below :—

Pseudohypertrophic muscular dystrophy; Hydatidiform mole; Kidney in sulphanimide poisoning; Gynaecomastism; Monckeberg's sclerosis of uterine arteries; Rhinosporidiosis; Lupus vulgaris; Tuberculosis of the breast; Nodular fibroma of the vocal cord; Guinea-worm abscess; Encephalocele of suboccipital region; secondary deposit of Chorionepithelioma in vagina and Mycetoma foot.

Post-graduate training in pathological histology was given to two Medical Officers deputed by the Baroda Government and the Principal, Medical College, Amritsar.

TABLE II.—*Showing the regional distribution of the malignant growths received during the year 1944*

	Uterus	Cervix	Eye-brow	Breast	Anus	Prostate	Neck	Testis	Ovary	Cheek	Knee	Palm	Hand	Scalp	Lymph nodes	Kidney	Total
Squamous-cell carcinoma	..	—	5	—	—	3	—	—	—	—	1	—	—	—	—	—	9
Basal-cell carcinoma	..	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
Adenocarcinoma	..	3	2	—	—	—	1	—	—	1	—	—	—	—	—	—	7
Carcinoma simplex	..	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	2
Carcinoma embryonal	..	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	2
Carcinoma medullary	..	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
Carcinoma secondary	..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4
Hypernephroma	..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Sarcoma	..	—	—	1	—	—	—	—	—	—	1	1	1	1	—	—	5
Malignant lymphoma	..	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
Total	..	3	7	2	2	3	1	1	2	2	1	1	1	1	1	4	33

TABLE III.—*Showing the regional distribution of the*

	Anus	Breast	Thigh	Cervix	Bladder	Labium	Heel	Vagina	Prostate	Neck	Jaw	Uterus	Axilla
Squamous-cell carcinoma	5	—	1	2	—	—	2	1	—	—	1	—	—
Adenocarcinoma	—	1	—	—	—	—	—	—	2	—	—	2	—
Carcinoma simplex	—	2	—	—	1	—	—	1	—	—	—	—	—
Medullary carcinoma	—	1	—	—	—	—	—	—	—	—	—	—	—
Scirrhus carcinoma	—	6	—	—	—	—	—	—	—	—	—	—	—
Papillary carcinoma	—	—	—	—	1	1	—	—	—	—	—	—	—
Chorionepithelioma	—	—	—	—	—	—	—	—	—	—	—	1	—
Secondary carcinoma	—	—	—	—	—	—	—	—	—	5	—	—	2
Seminoma	—	—	—	—	—	—	—	—	—	—	—	—	—
Osteogenic sarcoma	—	—	—	—	—	—	—	—	—	—	1	—	—
Fibrosarcoma	—	—	1	—	—	—	—	—	—	—	1	—	—
Lymphosarcoma	—	—	—	—	—	—	—	—	—	1	—	—	1
Melanotic sarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—
Hodgkin's sarcoma	—	—	—	—	—	—	—	—	—	1	—	—	—
Total	5	10	2	2	2	1	2	2	2	7	3	3	3

malignant growths received during the year 1945

[illegible]

TABLE IV.—*Showing the regional distribution of the*

	Breast	Liver	Cheek	Vulva	Lip	Jaw	Parotid	Ear	Thyroid region	Waist	Foot	Rectum	Uterus	Scrotum
Squamous-cell carcinoma	2	—	3	1	1	1	—	1	—	1	2	—	—	1
Scirrhus carcinoma	11	—	—	—	—	—	—	—	—	—	—	—	—	1
Bileduct carcinoma	—	1	—	—	—	—	—	—	—	—	—	—	—	—
Carcinoma simplex	1	—	—	—	—	—	1	—	1	—	—	—	—	—
Adenocarcinoma	—	—	—	—	—	—	—	—	—	—	—	1	3	—
Papillary adenocarcinoma	—	—	—	—	—	—	—	—	—	—	—	—	5	—
Basal-cell carcinoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carcinoma medullary	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Alveolar carcinoma	1	—	—	—	—	—	1	—	—	—	—	—	—	—
Mucoid carcinoma	2	—	—	—	—	—	—	—	—	—	—	—	—	—
Choriocarcinoma	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Seminoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carcinoma secondary	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Fibro sarcoma	—	—	—	—	—	—	1	—	—	—	—	—	—	—
Lympho sarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Round-cell sarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Neurogenic sarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Spindle-cell sarcoma	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Osteogenic sarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hodgkin's sarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	18	1	3	1	1	1	3	1	1	1	2	2	9	1

malignant growths received during the year 1946

Tongue	Forehead	Penis	Neck	Perineum	Face	Abdomen	Anus	Ovary	Vagina	Testis	Nostril	Maxilla	Tibia	Thigh	Leg	Wrist	Back	Hand	Hip	Total
2	—	1	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	18
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	5
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
—	3	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2
—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4
—	—	—	—	—	—	1	—	—	—	—	—	1	1	1	—	1	—	1	1	8
—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	2
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	2
—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
2	3	1	7	2	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	76

XI. Department of Virus Diseases including Rabies

During the years under review, 13,596 persons attended for anti-rabic treatment at the Institute. Out of these, 11,862 completed the full course of treatment, and 31 discontinued treatment: the rest were advice cases, in which the offending dog not suspected to be rabid, the persons bitten were given only 2 injections, if the dog proved not rabid after keeping under observation for a period of 14 days.

One case developed hydrophobia in 1944 after prophylactic treatment. The patient, who had received two punctured wounds on the hand as a result of a bite of a stray dog, sought treatment 45 days later, when the wounds had all healed up. Consequently, class III treatment for 14 days was given. The patient developed hydrophobia 4 days after the completion of the course of treatment. The failure of prophylactic treatment in this case was obviously due to the very long period that elapsed between the bite and the commencement of treatment.

As a measure of combating the incidence of canine rabies in the City of Bombay, the Municipality on the initiative of this Institute introduced the system of licensing ownership of dogs, to facilitate the systematic destruction of stray dogs. It was expected that this measure would reduce considerably the incidence of canine rabies in the city; but the figures for persons treated at this Institute (Table I) do not appear to bear out these expectations materially. The number of dogs' brains received at this Institute for examination for rabies, however, shows a progressive decline from 1944 to 1946 (Table II). This fact may testify to an extent the enforcement of the measure; but the large number of stray dogs that are still seen daily roaming about indicate the need for greater attention on the part of municipal authorities.

A total of 26,537 anti-rabic treatments were supplied to different centres, and of these 1,865 were issued for veterinary use.

The following new anti-rabic treatment centres were opened during the period under review, bringing the total number of out-station centres to 117.

1. Padmakunvarbai Hospital, Rajkot.
2. State Hospital, Vijaynagar.
3. Civil Hospital, Bilaspur (C. P.).
4. G. T. Hospital, Bombay.
5. J. J. Hospital, Bombay.
6. St. George's Hospital, Bombay.
7. Government Dispensary, Nasik.
8. State Hospital, Jaora State.
9. Municipal Dispensary, Basein.

The President of the Margao Municipality was supplied with 3,000 doses of canine prophylactic vaccine (25 per cent glycerinated phenol vaccine) in 1944, 2,250 doses in 1945 and 600 doses in 1946.

TABLE I.—*Showing the anti-rabic treatment carried out at the Institute and the out-station centres*

Details					1944	1945	1946
Number of persons applied for treatment	...	...	...	...	4,578	4,853	4,165
Advice cases	...	...	...	...	516	481	706
Fully treated	...	...	...	...	4,031	4,372	3,459
Discontinued treatment	...	...	...	...	31	<i>Nil</i>	<i>Nil</i>
Percentage of health returns	...	...	...	...	98·7	97·8	Awaited
Cases of hydrophobia	...	...	...	...	1	<i>Nil</i>	Awaited
Mortality rate	...	...	...	...	0·02	<i>Nil</i>	Awaited
Number of anti-rabic centres	...	...	...	...	108	111	117
Number of treatments sent to outcentres	...	...	...	...	8,335	8,947	9,255
Number of treatments sent to the Veterinary Surgeons	...	...	...	...	584	649	632

TABLE II.—*Showing the number of brains received and examined at the Institute for evidence of rabies*

Details		1944		1945		1946	
		Number	Per-centage	Number	Per-centage	Number	Per-centage
Positive for rabies (Negri bodies present)	...	100	69·0	76	58·4	64	71·1
Negative for rabies (Negri bodies absent)	...	40	27·6	36	31·8	24	26·7
Received in a state unfit for examination	...	5	3·4	1	0·8	2	2·2
Total		145		113		90	

TABLE III.—*Showing the source of brains received and examined for rabies*

	1944				1945				1946			
	Positive	Negative	Unfit for examination	Total	Positive	Negative	Unfit for examination	Total	Positive	Negative	Unfit for examination	Total
Bombay City and Suburbs ..	48	23	0	71	42	20	0	62	33	10	0	43
Out-stations ..	52	17	5	74	34	16	1	51	31	14	2	47
Total ..	100	40	5	145	76	36	1	113	64	24	2	90

TABLE IV.—*Showing the classification of different animals with the results of their brain examination for rabies*

Animal	1944				1945				1946			
	Positive	Negative	Unfit for examination	Total	Positive	Negative	Unfit for examination	Total	Positive	Negative	Unfit for examination	Total
Dog ..	92	33	2	127	73	32	1	106	61	18	2	82
Cat ..	—	3	—	3	1	3	—	4	1	1	—	2
Cow ..	1	—	—	1	—	—	—	—	—	—	—	—
Buffalo ..	—	—	3	3	—	—	—	—	1	—	—	1
Bullock ..	3	—	—	3	—	—	—	—	—	2	—	2
Deer ..	—	1	—	1	—	—	—	—	—	—	—	—
Fowl ..	—	1	—	1	—	—	—	—	—	—	—	—
Fox ..	1	—	—	1	—	—	—	—	—	—	—	—
Goat ..	1	—	—	1	1	—	—	1	1	—	—	1
Horse ..	—	2	—	2	—	1	—	1	—	1	—	1 (mare)
Man ..	2	—	—	2	—	—	—	—	—	—	—	—
Jackal ..	—	—	—	—	1	—	—	1	—	—	—	—
Rabbit ..	—	—	—	—	—	—	—	—	—	1	—	1
Total ..	100	40	5	145	76	36	1	113	64	24	2	90

TABLE V.—*Showing the number of brains received from different sources*

Received from	1944				1945				1946			
	Positive	Negative	Unfit for examination	Total	Positive	Negative	Unfit for examination	Total	Positive	Negative	Unfit for examination	Total
Bombay Veterinary College ..	28	15	—	43	30	15	—	45	18	5	—	23
Bombay Police ..	7	—	—	7	1	—	—	1	2	—	—	2
Bombay Private Owners ..	4	4	—	8	2	2	—	4	7	4	—	11
Other Sources in Bombay ..	5	2	—	7	5	1	—	6	6	1	—	7
Out-stations ..	56	19	5	80	38	18	1	57	31	14	2	47
Total ..	100	40	5	145	76	36	1	113	64	24	2	90

Yellow fever inoculations.—Since 1942 the Haffkine Institute has been storing yellow fever vaccine, manufactured at the laboratories of the Rockefeller Foundation, on behalf of the Public Health Commissioner with the Government of India, New Delhi. The yellow fever vaccine is a dried preparation of the virus, known as 17-D, grown in the developing chick embryo. The vaccine contains living virus attenuated as a result of prolonged cultivation in tissue culture. As this vaccine rapidly deteriorates, when exposed to room temperature, it is stored continuously at temperatures not exceeding 2°C. For use, the dried vaccine is rehydrated in sterile normal saline. The potency of the vaccine is periodically determined by inoculating intracerebrally various dilutions into mice to ensure that a single human dose, viz., 0.5 c.c. contains not less than 750 mouse units.

In order to control the spread of yellow fever, one of the steps taken by the various Governments concerned is to insist on passengers, proceeding from or by way of ports, in which yellow fever is reported to be present, taking inoculations of yellow fever vaccine. Over 12,000 inoculations have been carried out during the years under report.

XII. Blood Bank Department

The activities of this department have gradually increased during the years under report. The Government of Bombay started its own blood transfusion service in 1945, and took over the existing staff from the Red Cross processing unit at this Institute, to which was attached a mobile blood collecting unit for the collection of blood from voluntary donors in the various jails of this province.

The main sources of blood were the Indian Red Cross Society Blood Collecting Centre, the Civil Hospital Emergency Committee Blood Bank, which discontinued its activities from 31st August, 1946, Blood collecting Centre at this Institute and various jails in the province. In addition various city hospitals were sending

surplus blood from their local banks for processing into plasma. The blood was collected both on voluntary and payment basis. The payment to the donors was made from the proceeds of the sale of plasma. Table I gives the number of donors from different blood collecting centres.

TABLE I.—*Showing the number of blood donors*

Centre	Type of donors	Number of blood donors		
		1944	1945	1946
Indian Red Cross Society	Voluntary	—	978	168
	Paid	—	1,476	1,988
	Total	2,096	2,454	2,156
Civil Hospital Emergency Committee	Voluntary	—	174	136
	Paid	—	178	795
	Total	—	352	931
Haffkine Institute	Voluntary	—	—	427
	Paid	—	—	411
	Total	—	—	838
Other Hospitals		—	—	639
Grand Total		2,096	5,612	8,489

The citrated whole blood was processed into plasma, and was preserved by drying it from the frozen state. This was originally done on a plant constructed locally. The Strumia Type Blood Plasma Drying System Machine was installed in March, 1945, and this facilitated the lyophilizing to a great extent.

The blood was collected in sterilized Medical Research Council blood-collecting bottles, which contained 50 c.c. of sodium citrate sulphathiazole solution. About 250 to 300 c.c. of blood was collected in each bottle, which was tested for sterility on the next day. Kahn tests and blood groupings were done in every case. The blood was allowed to settle down, and the supernatant plasma was pooled by water suction pump in suitable jars. This plasma was tested for sterility both for aerobes and anaerobes. When passed, the pool was distributed into bottles of 250 c.c. each and lyophilized. This final product was tested for sterility again as before and for toxicity by injecting intraperitoneally in 20 g. white mice. The moisture content of the dried product was also determined and was found to be constantly less than 0·8 per cent.

The dried plasma was distributed to the various city and district civil hospitals on a free quota basis, and was also sold at Rs. 55 per bottle of 250 c.c.

Requisite quantity of pyrogen free distilled water was supplied with each bottle of plasma for reconstituting it. The demands for this product both from this province and outside were considerable, and it was not always possible to meet these demands fully, especially during the disturbances in the City of Bombay. Tables II and III give the yield and distribution of blood and plasma.

TABLE II.—*Showing the blood and plasma yield*

Product	Quantity in litres		
	1944	1945	1946
Blood	395·67	703·47	1136·35
Liquid plasma	156·49	348·80	693·75
Dried plasma	—	331·26	515·75

TABLE III.—*Showing the distribution of plasma*

Nature	Quantity in litres		
	1944	1945	1946
Free	102·00	140·50	438·25
Paid	93·75	90·75	176·00
Total	195·75	231·25	663·25

Polyvalent antivenene was also lyophilized on the same machine in 10 c.c. ampoules. The batches of medical officers of the Government of Bombay and other private practitioners attending the resuscitation courses at the J. J. Hospitals attended the demonstrations on the working of this department.

XIII. Training of Health Personnel

This Institute plays an important part in dissemination of scientific knowledge by affording facilities to persons desirous of learning different types of techniques either for the prosecution of researches or for routine work in their different spheres of activities. In addition short courses of training of a preliminary nature are given to public health personnel with a view to enable them to carry on their disease control work efficiently. As a rule, most of the workers are from this country; but even persons from other far off countries are attracted to this Institute. The details of important and advanced types of training are mentioned

the department concerned; but the names of persons along with periods of training and the department in which they worked, are given below. In addition, a very large number of workers come to the Institute for casual help, as they are too many, their names are not mentioned in the following table :—

Serial No.	Name	Address	Department in which trained	Duration of Training
1	Mr. V. N. Bhargava, B.Sc.	King Edward Hospital and Medical School, Indore	Biochemistry	.. 13th March to 12th June, 1944.
2	Mr. Shankar Murthy, B.Sc.	Travancore Government Scholar	Clinical Pathology	.. 9th August, 1944 to 12th March, 1945.
3	Dr. R. R. Ghare Khan	.. Medical Officer, Baroda State	Clinical Pathology and Vaccine	.. 10th October, 1944 to 7th February, 1945.
4	Mr. V. R. Mudeunavar	.. C/o Public Health Department, Bombay, Poona	Entomology	.. 11th to 18th October, 1944.
5	Major B. N. Khan, I.M.S.	.. Chief Health Officer, New Delhi	Entomology	.. 5th to 12th March, 1945.
6	Dr. D. J. Chavan, M.B.B.S.	.. Devgad Baria	Virus Diseases	.. 10th to 16th March, 1945.
7	Dr. Thomas Darapar, Ph.C., F.R.C.P. (Edin.)	.. Canadian Presbyterian Mission, Jobat, C. I.	Pharmacology	.. March, 1945.
8	Dr. G. K. Apte, M.B.B.S.	.. Chief Medical Officer, Sangli	Virus Diseases	.. 10th to 23rd April 1945.
9	Dr. Liu Chun Hsiang	.. The National Epidemic Prevention Bureau, Kunming, China	Serum	.. 15th April to 15th May, 1945.
10	Mr. Chan Chiu Shuni	.. The National Epidemic Prevention Bureau, Kunming, China	Serum	.. 23rd May to 25th September, 1945.
11	Dr. Abdul Jaffar, B.Sc., M.B., Ph.D. (Edin.)	.. Lecturer in Physiology, Robertson Medical School, Nagpur, C. P.	Nutrition and Experimental Pathology and Biochemistry.	.. 26th May to 30th June, 1945.
12	Mr. P. D. Dalvi, M.Sc., F.I.C.	.. State Chemist, Jaipur	Biochemistry	.. June, 1945.
13	Mr. K. Velukutty	.. C/o Director of Public Health, Trivandrum	Entomology	.. 4th to 11th January, 1945.
14	Mr. V. M. Padmanabha Panikar	.. C/o Director of Public Health, Trivandrum	Entomology	.. 4th to 11th January, 1945.
15	Dr. Ali Mohammed	.. Assistant Professor of Pathology, Graney Medical College, Amritsar	Nutrition and Experimental Pathology	.. 2nd to 28th July, 1945.
16	Dr. Tiagi	.. Reader in Pathology, Medical College, Agra	Clinical Pathology	.. 3rd July to August, 1945.
17	Dr. K. V. Venkatraman, M.D.	.. Assistant Director, King Institute, Guindy, Madras	Vaccine	.. 28th August to 18th September, 1945.
18	Mr. C. C. Wu	.. Technical Expert, Parasitology Laboratory, National Institute of Health, Koloshan, Chungking, China	Entomology	.. October, 1945.
19	Mr. T. Velandapillai	.. Bacteriological Institute, Colombo	Serum	.. 4th February, 1946.
20	Dr. Mohammad Mussa Khan, D.P.H.	.. Nutrition Officer, Public Health Department, Punjab	Nutrition and Experimental Pathology	.. 23rd March to 3rd May, 1946.
21	Dr. R. Y. Rajopadhye	.. Pandharpur Municipality	Entomology	.. Four weeks from 27th April, 1946.
22	Rai Sahib Dr. Kailas Singh	.. K. E. Hospital & High School, Indore, C. I.	Blood Bank	.. 9th to 20th May, 1946.
23	Dr. N. D. Shah, M.B.B.S.	.. Chief Medical Officer, Lunawada State	Virus Diseases	.. 18th to 24th May, 1946.
24	Dr. V. P. Desai, L.M. & S.	.. Medical Officer, Vijaynagar	Virus Diseases	.. 11th to 17th June, 1946.
25	Mr. H. Riberio	.. Pathology Section, Grant Medical College, Bombay	Vaccine	.. August, 1946.
26	Dr. J. Lobo, M.B.B.S.	.. Medical Officer, Distillery Dispensary, Nasik Road	Virus Diseases	.. 23rd to 28th September, 1946.
27	Dr. H. Mirdamadi	.. Professor of Medicine, Tehran University	Serum and Clinical Pathology	.. For 6 months in 1946.
28	Major S. L. Kalra	.. Typhus Research Unit, Central Military Pathology Laboratory, Poona	Entomology and Clinical Pathology	.. 7 days in June, 1946.

XIV. Critics Club

The activities of the Critics Club were revived from 4th February, 1946. Weekly meetings are held every Friday, in which current literature on Scientific subjects is reviewed by the members of a department, and once a month a brief account of the original researches is given by a member. The rules for the functioning of the Critics Club are framed. The position of the club through the efforts of its secretary Dr. D. W. Soman is definitely established, and is functioning systematically, except for about six weeks during September and October when no meetings could be held due to the disturbed conditions in the city.

During the year 1946, 32 meetings were held, at which abstracts of current literature pertaining to various subjects were read by the members of the different departments, and at seven meetings the below-mentioned researches were reported. Farewell functions to the departing members, Dr. S. R. Savoor, Dr. H. S. Mahal and Dr. B. B. Dikshit, were held during the year 1946.

1. "Casein-hydrolysate Cholera Vaccine" by Maj.-Gen. Sir Sahib Singh Sokhey on 26th April, 1946.
2. "Prognostic Significance of Leucocytic Count in Bubonic Plague" by Dr. P. M. Wagle on 31st May, 1946.
3. "Prothrombin Time in Normal Indians" by Miss B. M. Braganca and Dr. M. V. R. Rao on 28th June, 1946.
4. "A Method of Improvement of Solubility of dehydrated Concentrated Horse Serum-Globulin by Addition of Sodium Taurocholate and Extraction with Ether" by Dr. D. C. Lahiri on 26th July, 1946.
5. "Impressions of his recent Tour in the U. S. A. and United Kingdom" by Maj.-Gen. Sir Sahib Singh Sokhey on 15th November, 1946.
6. "Use of Tolu-balsam in Ramon's Flocculation Test" by Dr. S. P. De on 29th November, 1946.
7. "Anti-allergic Serum" by Dr. D. C. Lahiri on 27th December, 1946.

In addition, some members of the Critics Club took part in four meetings of the Teaching Pathologists held at the Haffkine Institute. The following papers were read by them at these meetings during the three years under review :—

1. "Toxic Factors in Shock" by Dr. B. B. Dikshit on 24th June, 1944.
2. "Tsutsugamushi Fever" by Dr. S. R. Savoor on 24th June, 1944.
3. "Phrynoderma" by Dr. M. V. R. Rao on 18th November, 1944.
4. "Sulpha Drugs in the Treatment of Plague" by Dr. P. M. Wagle on 18th November, 1944.
5. "New lyophilized Polyvalent Anti-snake-venom Serum" by Dr. D. C. Lahiri on 28th July, 1945.
6. "On the Isolation of *V. cholerae* and the Serological Subtypes met with in Bombay" by Dr. D. W. Soman on 28th July, 1945.

7. "Prothrombin Time in normal Indians" by Miss B. M. Braganca on 29th June, 1946.
8. "A serological Test diagnostic of Plague Infection in Rodents" by Dr. J. P. Menezes on 29th June, 1946.
9. "Vaccine-lymph and some Immunological Problems connected with Vaccination" by Dr. R. Sanjiva Rao on 29th June, 1946.

XV. Income and Expenditure

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

1943-44

Receipts	Expenditure
Rs.	Rs.
XXVII—Medical—	38—Medical—
Sale proceeds of sera, vaccines and miscellaneous receipts ... 8,45,986	H-Bacteriological Laboratories—
	H-1 Haffkine Institute,
	H-2 Pasteur Institute,
	F-Special Development Programme—
	F-2 Research Scholarships,
	F-3 Scheme for serum manufacture—
	Pay of Officers ... 1,20,047
	Pay of establishment ... 1,24,838
	Allowances, etc. ... 46,503
	Contingencies ... 5,06,002
Total receipts ... 8,45,986	Total expenditure ... 7,97,390

1944-45

Receipts	Expenditure
Rs.	Rs.
XXVII—Medical—	38—Medical—
Sale proceeds of sera, vaccines and miscellaneous receipts ... 10,46,416	H-Bacteriological Laboratories—
	H-1 Haffkine Institute,
	H-2 Pasteur Institute,
	F-Special Development Programme—
	F-2 Research Scholarships,

1944-45—contd.

Receipts	Expenditure
Rs.	Rs.
	F-3 Scheme for serum manufacture—
	Pay of Officers ... 1,36,639
	Pay of establishment ... 1,33,377
	Allowances, etc. ... 81,855
	Contingencies ... 7,04,425
	Total expenditure ... 10,56,296
	Less—
	Recoveries from Defence Department for testing drugs ... 22,805
Total receipts ... 10,46,416	Net expenditure ... 10,33,491

1945-46.

Receipts	Expenditure
Rs.	Rs.
XXVII—Medical—	38—Medical—
Sale proceeds of sera, vaccines and miscellaneous receipts ... 18,96,606	H—Bacteriological Laboratories— H-1 Haffkine Institute, H-2 Pasteur Institute, F—Special Development Programme— F-2 Research Scholarships, F-3 Scheme for serum manufacture—
	Pay of Officers ... 1,46,414
	Pay of establishment ... 1,56,859
	Allowances, etc. ... 1,40,841
	Contingencies ... 7,43,417
	Total expenditure ... 11,87,531
	Less—
	Recoveries from Defence Department for testing drugs ... 23,103
Total receipts* ... 18,96,606	Net expenditure* ... 11,64,428

* The grant for making vitamin tablets is not included on either side.

PART II

RESEARCH WORK

I. Casein Hydrolysate Direct Cholera Vaccine

(SOKHEY AND HABBU)

The standard cholera vaccine was prepared from growth on agar slopes. The protective value of this vaccine was in dispute. Recent work showed that it did not possess significant protective value, but still not of a very high order. We took up work to prepare a cholera vaccine in a liquid medium.

We have successfully prepared a direct cholera vaccine which, by biological assay described below, is shown to possess 10 times the protective value of the agar-grown standard vaccine. Up to now, over 15 million cubic centimetres of the casein hydrolysate direct cholera vaccine have been used. We give below an outline of the method of preparation.

We found Mueller and Johnson's casein hydrolysate an excellent medium for the growth of cholera vibrios. The medium is made up to contain 135 mg. of nitrogen per 100 ml., and 600 ml. of this medium are dispensed in 3-litre modified Haffkine flasks. They are seeded with virulent strains of both subtypes, Inaba and Ogawa. Only such strains are used as kill at least 5 out of 10 mice intraperitoneally inoculated with 1,000 organisms. The culture is incubated at 37°C. for 3 days and killed by the addition of 0.1 per cent formalin. Phenyl mercuric nitrate, 1.5 mg. per 100 ml. is used as a preservative. The illustrative measurements of the protective power of cholera vaccines are detailed below :—

Mouse protective dose

I. Casein hydrolysate direct cholera vaccine No. 22—

(a) Tested against Inaba	...	...	0.000086 ml.
(b) Tested against Ogawa	...	...	0.000097 ml.

II. Some vaccines in use in India—

1. C121/44-8A

(a) Tested against Inaba	...	...	0.0012 ml.
(b) Tested against Ogawa	...	...	0.0360 ml.

2. 467/4

(a) Tested against Inaba	...	...	0.0027 ml.
(b) Tested against Ogawa	...	...	0.0004 ml.

3. No. 165845

Tested against Inaba	...	...	0.0038 ml.
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III. Testing of a vaccine against the same strain from which it was made and against two other strains—

1. Vaccine No. 81 (a) made from strain 1A (Ogawa)

(a) Tested against strain 1A (Ogawa)	...	0.000021 ml.
--------------------------------------	-----	--------------

(b) Tested against strain 2 Ogawa)	...	0·000025 ml.
(c) Tested against strain 3 (Ogawa)	...	0·000020 ml.

IV. Vaccines produced from avirulent cholera vibrio strains—

1. Agar suspension vaccines made from

(a) Strain 3 (Inaba); tested against strain 569B (Inaba)		0·0053 ml.
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(b) Strain 169 (Ogawa); tested against strain 1 (Ogawa)		0·18 ml.
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2. New casein hydrolysate vaccines

(a) Strain 3 (Inaba); tested against strain 569B (Inaba)		0·00074 ml.
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(b) Strain 169 (Ogawa); tested against strain 1 (Ogawa)		0·14 ml.
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The measurements of the virulence of some of the strains mentioned above in categories III and IV are given below :—

Strain	No. of organisms	Mice used	
		No.	Died
1A (Ogawa)	910	10	6
2 (Ogawa)	1,040	10	5
3 (Ogawa)	1,120	10	6
3 (Inaba)	1,080,000	10	0
3 (Inaba)	10,800,000	10	1
169 (Ogawa)	920,000	10	0
169 (Ogawa)	9,200,000	10	3

II. Biological Assay of Cholera Vaccine

(SOKHEY AND HABBU)

The making of a new vaccine demanded a reasonably reliable laboratory test for measuring the protective value of the vaccine. The current agglutination titre determinations of sera of animals immunized with a given vaccine was not a reliable measure. It was considered desirable to develop an active immunity test, something on the lines of our method for the biological assay of plague vaccines. We selected white mouse as the test animal; for Griffiths observed that white mouse is killed by intraperitoneal injection of cholera vibrios.

We have been successful in developing the desired test. Repeated determinations give very close results. An outline of our method is given below.

White mice, weighing 18–21 g., are used as the test animal. Strains of cholera vibrios for the challenge dose are selected on the basis that 1000 organisms, contained in 0·5 ml. of 5 per cent mucin suspension, when given intraperitoneally will kill not less than 5 out of 10 mice. The test infective dose is 100 times the minimum lethal dose of the selected strain of virulent vibrios.

The object was to determine the minimum amount of a given cholera vaccine which will protect 50 per cent of the animals against the standard test infective dose given intraperitoneally.

Method.—For any given vaccine, five or six graded doses arranged in a geometrical progression with a common ratio of 2 are decided upon after a preliminary test, so that the 50 per cent protective dose lies about the middle of selected series of doses. For each dose a batch of 6 mice, weighing 10–12 g., is used. The selected doses are given subcutaneously in two equal halves with an interval of seven days. First half of each dose is given and seven days later the second half is given. Seven days after the second half of the dose, the standard test infective dose is given intraperitoneally. At the same time, standard test infective dose is given to 6 mice weighing 18–21 g. The mice to which the vaccine had been given will have also grown to this weight in two weeks. The mice are observed for 48 hours.

If a vaccine under test is a mixed vaccine of both Ogawa and Inaba sub-types, as is usual, two sets of animals should be immunized and tested against Ogawa and Inaba, standard test infective doses, separately. Otherwise the vaccine should be tested against the standard test infective dose of the homologous sub-type.

Evaluation of the Results.—From the results obtained, the 50 per cent end-point is calculated by the method of Reed and Muench, (1938, *Amer. Jour. Hyg.* 27, p. 493) or some other equally suitable statistical method. One batch of vaccine tested 7 times gave the following results:—

					Mouse protective dose
1st Test	...	...	...	...	0·000028 ml.
2nd Test	...	...	...	...	0·000026 ml.
3rd Test	...	...	...	...	0·000050 ml.
4th Test	...	...	...	...	0·000041 ml.
5th Test	...	...	...	...	0·000040 ml.
6th Test	...	...	...	...	0·000025 ml.
7th Test	...	...	...	...	0·000049 ml.
Average 50·0 per cent mouse protective dose	...	...	...	...	0·000037 ml.
Standard deviation of the mean	...	...	...	...	0·00001 ml.

III. Casein Hydrolysate Direct Plague Vaccine

(SOKHEY AND HABBU)

We have worked out the conditions under which a very much improved plague vaccine with a consistently high protective value can be prepared by the use of Mueller and Johnson's casein hydrolysate. The medium is so prepared that controlled digestion produces a fluid entirely free of proteins, is perfectly limpid and has a light yellow tinge. It is adjusted to contain 270 mg. of nitrogen per 100 ml. This medium is distributed in quantities of 1 litre in modified Haffkine flasks of 3 litre capacity. The medium is seeded with specially selected strains of the plague bacillus preserved by drying from the frozen state. The culture is incubated at 28°C. for two weeks. It is killed by the addition of 0·1 per cent formalin. Phenyl mercuric nitrate is used as the preservative.

The mouse protective dose of this vaccine is 0.004 ml. It is much less toxic than our previous vaccine, as 0.6 ml. of it is needed to kill a mouse, while 0.2 ml. of the previous vaccine was lethal to a mouse. Its keeping qualities are very good; stored at 37°C. for 18 months does not show any loss of protective power.

IV. Chemotherapy of Plague

(SOKHEY, HABBU AND RAJAGOPALAN)

The new antibiotic streptomycin was found to be active against some gram negative organisms, particularly *Pasteurella tularensis*, the causative organism of tularemia. We have tried its action in the treatment of experimental plague and have found it to be very effective.

The drug was given subcutaneously either 48 or 72 hours after infection. The quantity of the drug was contained in 0.2 ml. of the fluid. The details of the experiments are given below:—

Dose	Hours after infection when drug administration started.	Number of organisms in the challenged dose.	Test animals		Control animals	
			No.	Died	No.	Died
0.2 mgs. four times daily for 7 days ...	48	95	10	3	12	12
0.2 mgs. four times daily for 7 days ...	72	140	10	5	12	12
0.4 mgs. four times daily for 2 days followed by 0.2 mgs. four times daily for the next 5 days ...	48	136	10	0	10	10
0.4 mgs. four times daily for 2 days followed by 0.2 mgs. four times daily for the next 5 days ...	48	124	10	1	10	10
0.8 mgs. four times daily for 2 days followed by 0.2 mgs. four times daily for the next 5 days ...	72	124	10	1	10	10

V. Rate of Growth of the Plague Bacillus

(SOKHEY AND HABBU)

Using the technique for counting colonies described elsewhere (Sokhey, 1939, *Ind. J. Med. Res.* 27, p. 331), we have studied the growth of the plague bacillus in the same medium as was used in our previous studies (Sokhey & Habbu, 1943, *J. Bact.*, 46, pp. 25-37). For these determinations also we used 10 ml. of the medium placed in test tubes having 1.7 cm. internal diameter. Inoculum was 0.1 ml. of 1 in 10 dilution of 48-hour growth of the organisms in the same medium. On an average, the inoculum had 600,000 organisms.

This study has yielded some significant results. It was noted that plague bacillus, when grown in a liquid medium, has two stationary phases of growth—first from 0 to 1 hour and the second from 21 to 24 hours. After each of these stationary phases, there is a logarithmic growth phase. During each of these logarithmic phases of growth, the generation time is 80 to 90 minutes.

This phenomenon appears to be due to the fact that the growth at first begins in the body of the medium, which has an oxygen content of 0.72 to 0.75 ml. per 100 ml. of the medium. This growth in the body of the medium comes to an end in about 21 hours, when the oxygen content has fallen to 0.04 ml. per 100 ml. During this period no growth takes place on the surface of the medium, but by the time the maximum growth has taken place in the body of the medium and a stationary period has set in, the CO_2 evolved by the organism lowers the oxygen tension on the surface of the medium, which permits growth on the surface of the medium.

This study has thrown fresh light on the nature of the initial stationary phase of growth. Several explanations have been offered for the occurrence of this phase. Our experiments indicate that the initial stationary phase and lag phase are caused by the lack of some nutritional factor in the medium. This fact is strongly borne out by an experiment in which we used a very small inoculum, i.e. 735 organisms per ml. of the medium. The initial stationary phase lasted for 78 hours as against 1 hour in our previous experiments. We noted that during this period, the number of organisms continued to decrease until by the end of 78 hours the number had fallen to 50 organisms per ml. Then the logarithmic phase of growth began giving a generation time of 106 minutes. It appears that the organism could not grow in the medium because some nutritional factor was lacking; but the death and dissolution of a number of organisms in the medium made good this nutritional deficiency and permitted the growth to proceed.

VI. An Anti-allergic Serum.

(D. C. LAHIRI)

The use of horse derived antitoxins in man has always been attended with certain amount of risk, such as "serum sickness", and "serum shock". These allergic reactions have tended to discourage the use of horse derived antitoxins. Several methods devised to alter or remove the reaction producing proteins by enzymatic digestion and complex chemical fractionation methods, have not proved entirely satisfactory. It is now recognized that "serum sickness" and "serum shock" are of the nature of specific antigen antibody reactions. The antibody in the human system, which reacts with horse serum proteins to produce allergic reaction, would be expected to be part and parcel of the human serum proteins. Hence it was thought that if human serum proteins were used as an antigen to immunize a horse, the immune horse serum would contain an antibody which, when injected into human body, would react and inactivate the allergy producing antibody fraction of the human serum.

For the same reason, anti-guineapig serum would be expected to prevent anaphylaxis due to the injection of horse serum into guineapigs previously sensitized with horse serum. This experiment, therefore, offered a method of testing the

procedure in an experimental animal. Thus, in addition to the direct tests of the procedure in man, experiments were conducted in guineapigs. The anti-guineapig serum was obtained by immunizing rabbits against guineapig serum.

1. *Experiments in man.*—For these an anti-human serum was prepared by immunizing horses with pooled human plasma. The anti-allergic properties of the anti-human horse serum were determined by three different methods: (1) by intracutaneous injection of the anti-human horse serum into one forearm and normal horse serum into the other forearm of the subject; (2) by subcutaneous injection of anti-human serum into one group of volunteers and normal horse serum into a second group of volunteers; and (3) by the subcutaneous and intramuscular injections of the therapeutic sera mixed with 5 g. of dried anti-human serum per litre of therapeutic serum.

In the case of the intracutaneous injection, the numbers and sizes of the wheals, and the extent of erythema in the respective forearms, suggested that the reactions due to the anti-human horse serum were much less intense than those due to the normal horse serum. In the case of subcutaneous injections, one group of 22 men were injected subcutaneously with 1 ml. of the anti-human horse serum. Five to ten days after the injection, five men developed allergic serum reactions; but they were very mild, and were noticed probably because the men had been told to observe carefully any appearance of reaction. The other group of ten men were injected subcutaneously with 1 ml. of the normal horse serum. Seven developed local reactions of varying severity. In two of these seven subjects, the reactions were later generalized and the men had to be treated with injections of adrenalin and local application of calamine lotion.

For experiments with therapeutic sera the dose of anti-human serum was fixed arbitrarily on the basis of preliminary safety tests. For safety tests, 5 ml. of the anti-human horse serum was given subcutaneously daily to a group of subjects for 20 days without any harmful results. Antiplague serum was specially selected for the experiment, because it is issued in the natural state and is administered in very large doses, and is known to cause allergic reactions in about 70 per cent of cases. During an outbreak of plague, 42 cases of bubonic plague were treated with the anti-plague horse serum, to which 5 g. of the dried anti-human serum per litre had been added. It was reported to us that 40 out of the 42 cases treated showed no allergic symptoms at all.

2. *Experiments in guineapig.*—Anaphylaxis, which is also an allergic reaction, and can be easily produced in guineapig, offered us an alternative method of testing the validity of our hypothesis. The anti-guineapig serum had, however, to be prepared in rabbits, because it was simply impossible to get litres of guineapig serum required for immunizing horses. The immune rabbit serum was, however, toxic for guineapig. The antibodies to the guineapig serum proteins had, therefore, to be purified from the toxic substances present in the immune rabbit serum. Purification of the anti-guineapig bodies from the rabbit serum was done by the simple procedure of flocculating out the antibodies with guineapig serum proteins. These floccules were used for determining the anti-allergic effect of the anti-guineapig serum.

The anti-guineapig rabbit serum floccules administered subcutaneously or intracardially prevented anaphylaxis in 21 guineapigs out of 22; two guineapigs out of the total number of 24 used for the test had died before the shock producing dose could be injected. Of the 21 animals which did not show anaphylaxis, four had developed "serum shock", but recovered from it. Of the

26 controls which had variously received non-specific floccules or normal guineapig serum, three had died before shock producing dose was given, 20 died of anaphylaxis. The remaining three animals which did not develop anaphylaxis, showed signs of serum shock, but recovered from the effects.

These results clearly demonstrate that anaphylaxis can be prevented in guineapig by the use of anti-guineapig rabbit serum floccules, and tend to confirm the results of experiments in man designed to prevent the allergic reactions by the use of anti-human horse serum. Thus, both the laboratory experiments and observations in man lend great support to the hypothesis that the anti-antibody would block the antibody responsible for producing allergic reactions in a subject. The practical procedure based on this hypothesis, i.e. the production and the use of anti-human serum is exceedingly simple and inexpensive, and offers an effective solution of the age-long problem of eliminating allergic reaction attendant on the use of horse derived antitoxins and sera. We feel that this procedure may have a much wider field of application, in that it may be useful in preventing all types of allergic reactions in man.

VII. Preparation of Polyvalent Anti-snake-venom Serum effective against the Venoms of Cobra, Russell's Viper, Common Krait and Phooras

(HAZRA)

Immunization of horses with each of the four venoms gave no higher response than immunizing horses with a mixture of all four of them. The second method had the distinct advantage that a serum of a given strength could be obtained more readily. We, therefore, decided immunizing horses with mixtures of venoms.

We keep our stock of venoms in lyophilized form, permitting us to use the venom in any desired dilution. It was, however, found that if venom is well diluted with physiological saline so that 1 mg. of crude venom is contained in 10 ml. of the dilution, the injection is never followed by any untoward reaction such as formation of abscesses or necrosis.

Immunization is begun with 0.25 ml. of a mixture of equal parts of 1 : 10,000 dilution of the venoms. This is followed a week later by 0.5 ml. of the same mixture. At weekly intervals, the dosage is doubled till a total of 1 mg. of venom is injected. The animal is rested for a month. Immunization is started again with 0.25 ml. of a mixture of equal parts of 1 : 100 dilution of the venoms. Subsequent injections are progressively doubled and injected at weekly intervals until 70-90 mg. of the total venoms are injected as a single dose. The process of immunization takes roughly three months in all. This immunization, on an average, gives a natural serum of which 1 ml. can neutralize 0.3 mg. of each of the four venoms. The natural serum is then concentrated with ammonium sulphate and lyophilized. The serum standardized according to the technique given in the paragraph below has neutralizing power of 0.6 mg. of each of the Cobra and Russell's Viper venoms and 0.45 mg. of each of the Krait and Echis venoms.

VIII. Standardization of Polyvalent Anti-snake-venom Serum

(HAZRA, LAHIRI AND SOKHEY)

Following the recommendations of Ipsen to the Permanent Commission on Biological Standardization of the League of Nations Health Organization, we

have worked out a method for standardizing this polyvalent serum. The method using white mouse as the experimental animal determines the relation between the antitoxin and the venom, irrespective of the level of test chosen. For this determination, standard venoms are employed. For each venom, the minimum lethal dose is determined. For preliminary determinations, 4 mice are injected with mixtures of 0.25 ml. of serum and 0.05 mg., 0.10 mg., 0.15 mg., and 0.20 mg., respectively of venom. The results are plotted on a graph, abscissa=mg. venom, and ordinate=ml. of serum. On this graph, a straight line is drawn separating the deaths from the survivals in the series of minimum lethal doses and the series injected with 0.25 ml. of the serum. Then four groups of 4 mice each are injected with mixtures of 0.20, 0.15, 0.10, and 0.05 ml. of serum with doses of venom chosen from the diagram, following the principle that the mixture of serum and toxin should have co-ordinates lying in the neighbourhood and on both sides of the preliminary line. Deaths and survivals of the mice are noted on the diagram and the final neutralization line drawn. The neutralizing power of the serum is calculated by selecting two points on the neutralization line, the difference of the corresponding amounts of the venom is divided by the difference of the corresponding amounts of the serum. Repeated determinations of the serum have, in our hands, given remarkably consistent results for each of the four venoms.

IX. Detoxication of Cobra Venom with Formalin and Heat

(HAZRA)

Grasset has advocated detoxication of venoms with formalin and heat. He was successful in immunizing horses with detoxicated venoms, which produced high titre anti-venene in a short time, without causing any local necrotic or inflammatory reactions. In view of good results obtained by Grasset with detoxicated venoms, attempts were made at this Institute to detoxicate cobra venom by his method described in *Trans. R. Soc. Trop. Med. Hyg.*, **38**, p. 463 (1945). Solutions of cobra venom were prepared in suitably buffered Martin's broth. The pH of the solution was adjusted to 7.3, and 0.8 per cent formalin was added; the formalized venom solution was incubated at 37°C. The sample was tested for toxicity in guineapigs, but neither the sample, which was collected after 30 days' incubation, nor the one, collected after 60 days, showed detoxication. Attempts in the past, with small samples of venom, have shown that with larger quantities of formalin and longer incubation at 37°C., the venom is detoxicated; but then it loses its antigenicity also. No success in detoxicating cobra venom by Grasset's method was obtained, without at the same time destroying its antigenicity. Further work is in progress.

X. Modified Digest Medium for producing High Titre Diphtheria Toxin

(DE)

For hyper-immunizing horses against diphtheria toxin, it is desirable to use only such lots as show high Lf values. After trials with different media, it was found that a pig-stomach-digest medium, which was prepared by a method modified from that recommended by Loiseau and Philipp (1939, *Ann. Inst. Past.*, **62**, p. 469), is the most suitable. The following modifications were made in the original formula, and the highest Lf values were obtained thereby. The amount of minced pig-stomachs was increased to 500 g. per litre and 1.5 per cent HCl was added.

to it. Digestion was carried out at 37°C. for 3 days, after adding a few ml. of toluol to prevent growth of organisms. Peptic digestion was followed by tryptic. For this purpose, supernatant fluid was decanted, pH was brought back to 8.2, and 10 per cent by volume of Cole's pancreatic extract was added. Digestion was allowed to proceed for 4 days at 37°C. Enzymes were then inactivated by heating and the digest was sterilized by filtration through Seitz. The digest was diluted with beef infusion in the proportion of three parts of digest to two of broth. After sterilizing the digest broth, 0.3 per cent maltose and 0.15 per cent glucose was added to it, just before inoculating it with *C. diphtheriae*. Toxins of satisfactory Lf values were obtained with this medium. The average Lf value was 25 units, and several batches even showed as high a value as 75 units.

XI. Determination of the Factors influencing the Time of Flocculation Kf

(DE)

It has been observed that during flocculation tests some samples of toxin-antitoxin mixtures flocculate very quickly, whereas others take a very long time. This is due to the rate at which the combinations between toxin and antitoxin takes place, and is supposed to be an inherent factor of the antitoxin. The term "avidity" is applied to this inherent quality of the antitoxins. Recently Byod called this phenomenon "E-optimum" reaction, and held both the toxin and the antitoxin responsible for it. Different series of experiments were set up, in which either of the two factors were varied and the influence of this variation on the time of flocculation were noted. Results, so far obtained, show that the antitoxin alone is not responsible for the "Kf". Further study is in progress.

XII. Modification of Ramon's Flocculation Test with Tolu-balsam Emulsion

(MIRDAMMEDI AND DE)

The difficulties encountered in Ramon's flocculation test for Diphtheria toxins and antitoxins are :—(1) the floccules are so fine, that they are liable to be missed by the inexperienced eye, (2) the time of flocculation is sometimes very much delayed, and (3) the discrepancy between the 'in vitro' and the 'in vivo' methods is sometimes very considerable. Investigation was undertaken to modify the test so that the above difficulties might be minimized. Several neutral agents were tried to bring about better binding between the antigen and antibody. It was finally found that emulsion of tolu-balsam stabilized with cardis-pipin (without cholesterol) served the purpose best. By incorporating a minute quantity of this reagent in the antigen-antibody system, it was possible to get (1) larger and easily visible floccules, (2) the time of flocculation was hastened, and (3) there was a better correlation between the "in vitro" and the "in vivo" results.

XIII. On the Pulicidal Effect of DDT

(SHARIF)

Experiments on determining the pulicidal value of dichloro-diphenyl-trichloro-ethane, popularly known as DDT, were carried out. It was used in the form of solution, emulsion and dust. Two types of experiments were conducted; one in

experimental jars, and the other in artificial, W-shaped, twenty-one feet long burrows simulating a natural rat burrow. Aqueous suspension containing 50 per cent DDT was also tried, and it was found useful for spraying floors.

For experiments with spraying, saturated solution of DDT in kerosene oil, and kerosene soap emulsion containing this compound, were used. Only experiments in jars were tried with sprays. Each jar containing 1,000 g. of sand, was at first sprayed with a measured quantity of the solution or of the diluted emulsion, and then shaken in such a way that its entire quantity was thoroughly mixed with the sand. About 0.08 g. of DDT in solution with kerosene oil gave 100 per cent kill of adult rat-fleas of *Xenopsylla cheopis* and *X.astia* in the experiments tried up to 120 days after spraying. Even 0.017 g. of DDT in the form of kerosene soap emulsion killed 100 per cent rat-fleas, so far, up to 49 days.

Delousing powder, 10 per cent DDT in pyrophyllite, U. S. Patent No. 2329074, was used for experiments with dusting. Both jar and burrow experiments were tried with it. In the former 12,799 fleas and in the latter 4,200 fleas were utilized. In order to ascertain the lethal effect of the DDT dust on rat-fleas, attention was paid to their behaviour under the influence of DDT, and its residual effect and minimum lethal dose for them; for these factors enable to determine the concentrations and quantities of DDT required for their control under diversity of situations in which they are found in nature.

DDT is a slow killing insecticide. When adult fleas come in contact with the DDT dust, the well-defined symptoms that occurred in succession in them are (1) excitement, (2) loss of jumping power, (3) loss of crawling power, and (4) stoppage of kicking of legs. Every lethal concentration of DDT killed all the fleas within 24 hours; but the rapidity with which earlier two symptoms occurred, depended upon its concentration. Thus the behaviour of fleas towards DDT could be utilized in assessing roughly the concentration of its different formulations in the field.

In the jar experiments, a concentration of 1 in 10,000 continued to kill 100 per cent rat-fleas up to 396 days tried after dusting the DDT powder. In the control experiments the mortality rate varied from 3 to 33 per cent. A very negligible deterioration in the immobilizing effect of DDT, as indicated by the delay in inhibition of the jumping power of all the fleas, with the length of exposure to natural elements occurred; but this was only confined to the uppermost layer of mixture of DDT dust and sand.

The minimum lethal dose for the adult fleas of *X.cheopis* was 0.005 mg. of DDT per gram of sand, as 99 per cent of them died 238 days after mixing it with the DDT dust. Thus even a concentration of 1 in 200,000 gave a fairly long residual effect for adult fleas; but higher concentrations only helped in the early abolition of their jumping power. A concentration of 1 in 300,000 was, however, not totally effective after 125 days.

In artificial burrows, when dusted with 37-79 g. of 10 per cent DDT powder with a calcid baby duster or by a cyanogas "A" foot pump duster, the results were not so consistent as in the jar experiments. In the individual experiments, the percentages of mortality on the total and the recovered fleas respectively were 58-100 and 97.8-100 within 428 days tried in the first arm up to a distance of about 5 feet, 68-96 and 81.3-100 within 425 days in the second arm up to a distance of about 10½ feet, 50-100 and 54.4-100 within 397-420 days in the third arm up to a distance of about 15½ feet, and 30-96 and 33.3-100 within 230-336

days in the fourth arm up to a distance of about $20\frac{1}{2}$ feet. The discrepancies met in the burrow experiments lend to reasonable analyses which incidentally throw light on the various difficulties likely to be encountered, whilst dusting natural rat burrows for flea control, and on the steps to be taken to overcome them in field operations.

Less than 100 per cent success in the mortality of fleas, obtained in many cases in the artificial burrows, is more due to the disturbing factors than to the deterioration of DDT on volatilization, as is clearly borne out by 100 per cent mortality of fleas in jar experiments for almost as long a period as in the burrow experiments. The disturbing factors were unequal distribution of the DDT dust by the ordinary dusters used, resulting in a very poor dispersal of the dust in the last two arms of the W-shaped burrows, and in not mixing of the dust with the finely powdered soil in them. Equal distribution of an adequate amount of the DDT dust in the rat burrow and its mixing with the burrow debris are very necessary. In order to do so the use of a power duster will be very helpful and economical.

In order to ascertain the effect of DDT on different stages of rat-fleas, 1 or 5 g. of the 10 per cent DDT dust were thoroughly mixed with 615 to 965 g. of debris of about one year old breeding jars, containing many thousands of adults, cocoons, larvae and eggs of *X.astia*. All the adults and larvae of the existing generation were killed within 24 hours. Only recently emerged adults and hatched larvae were seen alive, which ultimately died. Thus DDT is not ovicidal, and it has no effect on the pupae well protected within cocoons, but the adults and larvae are highly susceptible to it. On account of the residual effect of DDT the recently hatched larvae and emerged adults were killed off subsequently after coming in contact with the DDT dust.

XIV. On the Pulicidal Effect of "Gammexane"

(SHARIF)

Gammexane, the gamma isomer of benzene hexachloride generally known as 666, was tried in the form of emulsion and dust on the adult rat-fleas of *X.cheopis* and *X.astia*. A 5 ml. of diluted kerosene soap emulsion containing 0.004 g. of gammexane when sprayed on and thoroughly mixed with 1,000 g. of sand in a jar, i.e. a concentration on 1 in 250,000, killed 100 per cent adult fleas in an experiment tried up to 28 days.

Both the jar and burrow experiments were conducted with a powder containing 20 per cent of 666 with 2.6 per cent of gammexane. In the jar experiments 0.026 g. of gammexane, when mixed with 1,000 g. of sand, killed 100 per cent adult fleas up to 48 days tried after dusting. In the burrow experiments when dusted with 50 g. of this powder about 100 per cent adult fleas were killed 65 days tried after dusting the burrow at a place about $9\frac{1}{2}$ feet away from its entrance.

XV. On the Rodenticidal Effect of "ANTU"

(SHARIF)

Experiments to test the rat poison alpha-naphthyl-thiourea, generally known as "ANTU" "Chemical 109" or "Rat Poison 109", were carried out. In a concentration of 10 mg. per gram of the base of Bajri flour it was very effective for *Rat'us norvegicus* with a mortality rate of 94.1 per cent; but in this concentration it was ineffective for *R.rattus rufescens* and *Gunomys kok*, as the percentage of mortality in them was 10 and 6.7 respectively.

XVI. Typhoid Fever in the City of Bombay

(SOMAN)

During the three years under report, free diagnostic aid to private practitioners for the diagnosis of enteric infection was continued. The number of blood samples tested serologically definitely increased due to the presence of typhus infection in Bombay. As both these infections could not be clinically distinguished without difficulty, Widal and Weil-Felix reaction was done on every sample as a routine. The methods for the laboratory diagnosis of typhoid fever essentially remained the same as in previous years. The results as obtained by the combined methods of clot-culture and Widal reaction for the three years are given below in a tabulated form. No proved case of para-typhoid B and para-typhoid C infection was diagnosed either serologically or culturally.

Year			Total samples	Hospital samples	Private samples	Widal positive	Clot-cultures positive	
							B. typhosus	B. para-typhosus A
1944	...	...	1,738	1,325	413	488	184	20
1945	...	...	1,566	1,105	461	252	192	18
1946	...	...	1,561	1,097	464	249	185	14

Problem of contaminated clot-culture.—As no doctor was available at this Institute to collect blood for Widal tests and blood cultures from private cases, the medical practitioners used to collect the samples themselves and send to the Institute. As a result of this, the routine use of clot-cultures in OX bile presented a serious problem, for nearly 50 per cent of clot-cultures were found to be grossly contaminated by aerial and skin contaminants, such as staphylococci, gram positive sporebearing bacilli, *B. pyocyaneus*, etc. Under these circumstances, a necessity for finding some medium that would suppress the growth of contaminants without inhibiting the multiplication of typhoid bacillus was felt. Three different media, viz. bismuth sulphite broth, D. E. C. broth and glycerine peptone bile were tried in succession. The first two were modified Wilson and Blair's bismuth sulphite agar and D. E. C. agar respectively by substituting the agar base of both with the nutrient broth. The following table gives the results with different media for clot-cultures:—

Medium used		Total cultures	Percentage of conta- minated cultures	Percentage of sterile cultures	Percentage of positive cultures
OX-bile	...	3,422	47	38	15
B.S.B.	...	735	24	63	13
D.E.C.	...	335	25	62	13
Gly. Pep. Bile	...	1,562	20·9	65·6	14·5

B. S. B. = Bismuth sulphite broth ; D. E. C. = D. E. C. broth ; Gly. Pep. Bile = Glycerine peptone bile.

These results show that the percentage of contaminations has been reduced from 47 to 21, whilst the percentage of positive isolations has remained almost the same. Typhoid and para-typhoid A organisms could be grown easily in all the three media. Glycerine-peptone-bile, which is the cheapest and simplest of all the three media and has been proved superior to simple OX bile in reducing the chance of contaminations, was adopted for routine use of clot-cultures.

XVII. Serological Subtypes of *V. cholerae* isolated from Cholera Patients in Bombay

(SOMAN)

Observations on the serological subtypes of *V. cholerae* in relation to sporadic and epidemic outbreaks in India are very scanty. Finding no reference to subtypes prevalent in the City of Bombay and around, an opportunity was taken to investigate an outbreak of cholera in the city from this point of view. The outbreak continued for some months and was chiefly due to imported cases from adjoining infected districts. Over 1,000 faecal samples were examined bacteriologically and 151 strains of *V. cholerae* were isolated. D. E. C. medium was used throughout for cultural work. All these strains were typical cholera vibrios, giving a positive cholera-red reactions and non-haemolytic, and belonged to Heiberg's group I. They were agglutinable with cholera O, serum Group I, and were also tested with the mono-specific Ogawa and Inaba sera prepared at this Institute. Except one strain, which belonged to the Inaba subtype all the others were of the Ogawa subtype, and no "Intermediate" type was found. Inagglutinable vibrios were also isolated on a number of occasions.

XVIII. Paul-Bunnell Tests in the Diagnosis of glandular Fever

(SOMAN)

The test is based on the accidental discovery of a high concentration of heterophile antibodies against sheep erythrocytes in the serum of patients suffering from glandular fever. A preliminary survey of the natural heterophile agglutinins in 300 Wassermann sera in Bombay, revealed that agglutination in 1 in 80 dilution of a serum against sheep cells, could be taken as diagnostic in the presence of suggestive clinical findings. A further confirmation is obtainable by doing the absorption test in the serum with the guineapig kidney emulsion and beef cell suspension. The glandular fever antibody is not absorbed by the former but completely absorbed by the latter. During the years 1944 and 1945, 64 samples of blood, mostly from Royal Naval Hospital were submitted to Paul-Bunnell test and only seven of them gave serologically positive results. They were further confirmed by the absorption tests, following Barratt's modified technique. Besides, five clinically established cases were found to be negative on repeated serological examinations thereby showing the limitations of the test.

With a view to find out if any cases were being missed, 300 Widal sera were examined by the Paul-Bunnell tests and in only two titres reacted 1 in 80 and 1 in 160 respectively. Further absorption tests proved that the antibodies present were not of the glandular fever type. Absorption tests were, therefore, very valuable in excluding conditions like serum sickness or in confirming the border-line Paul-Bunnell results, in the diagnosis of glandular fever.

XIX. Value of Dye Test in the Rapid Diagnosis of Syphilis

(SOMAN)

The influence of a number of different dyestuffs on the flocculation test for syphilis was investigated by Berger who showed that Janus green and Victoria blue had both a sensitizing and indicator action on the reaction. Making use of this observation, he developed a simple and reliable slide flocculation test for the serodiagnosis of syphilis, recommending Victoria blue as the suitable dye in preference to Janus green. As this dye was not available at the time, Janus green was used in the test and 1365 samples of blood were tested in parallel by this dye test along with the routine Wassermann reaction. On comparing results, the dye test showed a fair degree of accuracy. A smaller series of blood samples were also tested by using Victoria blue in the dye test, and the results when compared with those of Wassermann reaction (577 samples) and Kahn tests (268 samples) showed 90 per cent accuracy in general and 95 per cent accuracy when only the negative reactions were considered. A fair trial was given to the Victoria blue dye test in the Military Laboratories during war and as a screen test, with which negative sera were eliminated and the positive sera were further tested by Kahn and Wassermann tests. The dye test seems a valuable screening test in a laboratory dealing with large number of samples.

XX. Preliminary Observations on a Six-hour Rat-test for the Diagnosis of Pregnancy

(SOMAN)

Since the twenty-four hour rat test was shown to compare very favourably with the classical hormonal tests for the diagnosis of pregnancy, many attempts have been made to shorten the period of test from twenty-four hours and to obtain reliable results. Experimental studies have shown that an immature white rat is equally sensitive to the hormonal response within a period of six hours, on the basis of which a six-hour rat test for the diagnosis of pregnancy was worked out. One rat was used for each test which was administered 4 ml. of urine subcutaneously once only. The rat was killed at the end of six hours and the result was noted and compared with the result of Friedman test or twenty-four hour rat test. One hundred samples of urine were thus tested, and the results described and analysed confirm the opinion that the white rat is a suitable experimental animal to replace the rabbit and that the test is rapid. A suggestion is made that a further trial be given to this test to assess its utility on a wider scale, as the rat offers certain and definite advantages over the mouse and the rabbit.

XXI. Studies on Leptospirosis

(SOMAN)

During the three years, 173 samples of blood were tested by the agglutination-lysis test, of which 13 were positive in a diagnostic titre of 1 in 100 or more. All the samples were taken from acute cases with jaundice of various duration of illness. Two of these samples were received from King Edward Memorial Hospital, one from the City Fever Hospital and one from Gokuldas Tejpal Hospital. Isolation of strains was only possible from the urine samples of these patients; but they were received only from two cases, and as such no strains could be isolated.

Of the sera sent for Widal reaction, 180 samples were examined by the agglutination-lysis test to find out whether any non-icteric type of cases were being missed. Only 2 samples showed a titre of 1 in 160 and 1 in 320 respectively. The former, when repeated after a week, showed no rise of titre; the latter came from a fatal case of typhoid bacteriologically proved. It was, however, presumed that the agglutinins in both cases were of the residual type derived from a previous leptospiral infection.

The leptospiral strains were kept up by subcultures in Vervoot's medium, though for maintaining the virulence, the strain had to be passaged through young guineapigs, approximately every four weeks. A very interesting observation was made on a culture in Vervoot's medium kept at room temperature on 28th February, 1945, which was derived from the heart-blood of an experimentally infected guineapig. When this culture was examined on 12th September, 1945, under dark ground microscope, only an organism was seen per field occasionally. 1.5 ml. of this was inoculated intraperitoneally into a young guineapig, which died on the 7th day of the disease, demonstrating that the virulence of the strain in Vervoot's medium remained unaffected for over a period of six months. This observation is very significant in view of the previous unsuccessful attempts of Lahiri to maintain the strains in a dried form from the frozen state.

A simple medium recently described by Gardner was tried for the growth of *Leptospira icterohaemorrhagiae*. It gave fairly abundant growth after 5 to 6 days. The medium was 12 per cent rabbit serum in freshly double-distilled water. It has been adopted in this laboratory as routine medium in preference to the old Vervoot's medium.

XXII. The Therapeutic Effect of some Compounds in Experimental Pneumococcus Type I Infection in Mice

(SOMAN AND MRS. VENKATRAMAN)

A few selected sulphanilamide compounds of the group thiazole and derivatives of 4-aminodiphenylsulphone with substituents in the 4'-position such as amino, the halogeno, hydroxy and cyano groups were tested against experimental pneumococcal infection in mice. Batches of ten mice, aged about 14 weeks and weighing 25-35 g., which were released after testing plague and cholera vaccines were infected with pneumococcus type I organisms; 0.5 ml. of 1:5000 dilution in 10 per cent serum broth were given intraperitoneally. The virulence and dilution were so standardized that all the control mice died of the infection in 24-48 hours. The drugs, suspended in gum acacia, were administered by mouth twice daily for five days and once on the sixth and seventh day in cases of survival. Daily mortality was recorded, and the experimental animals were kept under observation for a further period of 3-4 days. Sulphathiazole, which has been shown to give complete protection in a dosage of 20 mg., under the above conditions, was used as the standard drug for comparative evaluation.

Of the thiazole compounds, 2:4-dimethyl-5-sulphanilamidothiazole was found to give protection comparable with that of the standard in a similar dosage. In the case of some derivatives of diphenylsulphones the survival period was definitely prolonged even in small doses and mortality was usually delayed. The halogeno and cyano compounds of this series gave absolutely no protection in the dosage of 1 mg., the treated animals very often succumbing earlier than the controls. Higher dosage of 5 and 10 mg. seemed to prove quite toxic.

XXIII. Types of *C. diphtheria* in Bombay

(SOMAN)

This work was carried out from middle of October, 1945 to end of May, 1946. Nearly 500 throat swabs were examined and cultured for the presence of *C. diphtheria*, and 175 strains were isolated, out of which 167 were of the "mitis" type, 7 of "gravis" type and 1 belonged to "intermediate" type. Cultures were done on Leoeffler's serum and blood-tellurite medium. Out of 127 cultures thus isolated 93·7 per cent of the strains could grow on both media, 4 per cent only on blood-tellurite medium and 2·3 per cent on Leoeffler's serum, slope. On isolation, the morphology, colony appearance, haemolysin production and fermentation of usual substrates, in addition to starch and glycogen, were studied to determine the types. Virulence of 40 strains was tested in guinea-pigs by the intradermal test, and they were found to be virulent. One strain isolated from a cutaneous lesion from a patient was found to be avirulent.

On isolation of strains "chocolate starch medium" described by Hodgson and others was used for early determination of types. The results compared favourably with those obtained by fermentation of starch and glycogen which takes a longer time.

XXIV. Typhus in Bombay

(SOMAN)

The report is based on the summary of the work done during 1944, 1945 and 1946. During the first two years, Dr. Savoor and Dr. Vahia carried out the investigations. During the last year, the enquiry was conducted by Dr. Soman.

The types of typhus.—One of the useful methods of classification of typhus group of diseases is based on the agglutination reaction of the serum against organisms of the proteus X group, viz. X19, X2, XK. Weil-Felix reaction carried out on samples of blood sent for Widal tests or cultures has shown that the principal member of the typhus group of diseases present in Bombay is that corresponding to proteus X19. In this type, however, though agglutination reaction against proteus X2 strains occurs, it is in much lower titres. In 1945, there were two cases and in 1946 eight cases in which agglutination was mainly against proteus X2 and to a minor extent against proteus X19. Cases of scrub-typhus, so far, have been reported only from rural and not from metropolitan areas. In the six months commencing from June, 1945, eight cases were diagnosed, five of which were in individuals, who for a month prior to the onset of their illness, had never been outside municipal limits. It is of interest to note that two out of five cases showed an eschar, typical of the Tsutsugamushi disease. In 1946, 17 such cases were diagnosed; causal virus was demonstrated by animal inoculation test in 8 of them. Thus three serological types of typhus, corresponding to the three types of proteus X are met in Bombay.

Incidence of typhus.—The Executive Health Officer of the Bombay Municipal Corporation records 46 and 15 cases of typhus for the years 1944 and 1945; but according to our observations, 93, 110 cases respectively occurred. In 1946, 142 cases were diagnosed serologically. Obviously, all the cases of typhus have

not been notified to the Executive Health Officer. As endemic typhus has many clinical features resembling enteric group of diseases and the laboratory aid is not often sought for cases of continued fevers, it is possible that many cases of the former are clinically taken for the latter and notified as such. It is obvious, therefore, the number of cases of typhus in Bombay is much larger than is indicated even by the laboratory records.

Out of more than 200 cases of typhus serologically diagnosed at this Institute during the years 1944 and 1945, the symptoms of 63 hospital cases were studied by Savoor and Vahia. The prominent features were (1) headache present in all cases, (2) pyrexia of about two weeks' duration within the limits of 9-22 days, (3) rash in only 20 per cent of cases, (4) bronchial catarrh in about 25 per cent, and (5) low mortality. Only one case out of 63 proved fatal.

Epidemiology.—Typhus cases of the proteus X19 type occur throughout the year, the incidence being distinctly greater during the first six months. A sudden drop in the incidence occurs in June with the start of monsoon. Males and females of all communities, social classes and professions are equally affected. The peculiar incidence of typhus cases of proteus XK type, which occur mostly during the latter half of the year, especially in the months of September, October and November, has been noted during the years 1945 and 1946.

Serological diagnosis.—The minimum diagnostic titre for typhus infection was arrived at by submitting 1,263 samples of sera from febrile and afebrile cases to Weil-Felix test; 23 sera were positive in a titre of 1:125. As 98.2 per cent of sera were found to be negative in that titre, it was considered presumptive diagnosis of typhus infection and confirmatory, if suggestive clinical history was present.

To find out whether the normal Weil-Felix titre was influenced by disease of the enteric group, 174 sera from typhoid fever and 11 sera from para-typhoid fever cases, bacteriologically proved, were submitted to Weil-Felix test during different stages of disease. The result showed that the Weil-Felix titre was not affected by the enteric infection.

Isolation of Strains.—During 1946, 27 cases of typhus fever were serologically identified, and male guineapigs were inoculated with blood to isolate the rickettsial virus. Only in 11 cases, guineapigs showed a febrile reaction, and scrotal swelling (Neillmooser reaction) only in two cases. *Rickettsia* were demonstrated in four cases. The work of confirming the remaining cases by demonstration of *Rickettsia* or by other evidence is in progress. A murine strain of *Rickettsia* was recovered from Bombay rats, and when studied further by cross immunity experiments, using a known murine "Wilmington strain", the local strain was proved to be of the murine type.

Attempts have been made to isolate strains of *Rickettsia* from four cases giving Weil-Felix reaction with proteus OX2 in high titre. No strains were isolated.

Scrub-typhus.—In the previous report of the Institute, for 1942-1943, mention was made of the isolation of two strains of scrub-typhus from the Imphal and one from Calcutta. One more strain was isolated in the early months of 1944 from a patient coming from Ceylon. In 1945, two strains were isolated from patients in Bombay.

During the year 1946, 8 strains of scrub-typhus were isolated from patients in Bombay. The cases occurred during the months of September, October and November. White mouse was found to be the ideal animal for isolation of the strains. Usually four mice were inoculated intraperitoneally with 0.5 to 1 ml. of a ground blood clot suspension in physiological saline. The animal became ill within 8-14 days and the post-mortem examination showed typical findings, e.g. a gelatinous, sticky peritoneal exudate, an enlarged spleen, occasionally pleural exudate. Smears from peritoneal scrapings and stained with Giemsa, showed large number of *Coccobacillary* organisms within the cytoplasm of the serosal cells. The strains are maintained by serial passages in mice for further study.

Chemotherapy of experimental scrub-typhus in the mouse.—The mouse was selected as the animal of choice. Peritoneal fluid containing 10 M.L.D. of virus as the inoculum was given intraperitoneally 13 heterocyclic derivatives of diphenylsulphonates synthesized at the Haffkine Institute, were tried by Dr. Savoor, but none of them had any appreciable action on the course of infection in the mouse.

XXV. Action of Ions on the Central Nervous System

(PATEL)

Investigations on the action of ions on the central nervous system were continued on the same lines as reported before. A modification in the application of ions was introduced. This consisted in fixing a cannula in the lateral ventricles of anaesthetized animals which served as an inlet to the ion solutions and another cannula in the cisterna magna which served as an exit. Ion solutions varying between 1/5th and 1/10th molar were perfused and their action on the various systems of the body recorded graphically. It was found that NH_4 resembled K and Mg resembled Ca in producing their effects on the circulatory, gastro-intestinal and respiratory systems.

XXVI. Local Treatment of Infected Experimental Wounds

(PATEL)

Sulphonamides have been used very widely both locally and internally for the treatment of infected wounds. Local treatment is preferable to internal drug treatment, as it has the advantage of precluding the danger of severe toxic effects of the drug. With this idea in view investigation was undertaken to find out which of the six sulphonamides namely, sulphasuxidine, sulphadiazine, sulphaguanidine, sulphamethazine, sulphanilamide and sulphathiazole, gives best results in infected wounds.

Rabbits were used as the experimental animals for this investigation. Four circular wounds were made in shaved back of the rabbit. The wounds were infected with a drop of a suspension of the mixed culture of *Streptococcus haemolyticus*, *Staphylococcus aureus* and *Bacillus pyocyaneus*. The infected wounds were treated by application of a "Water-in-oil" type of emulsion made using cod-liver oil and various sulphonamides. Each animal had one wound which was not treated and served as a control. The animals were examined every third day and redressed.

Maximum concentration reached during the local application of the drug was determined by injecting the emulsion into the muscles of the flank. The concentrations of the drug never reached higher than 1.25 mg. per cent except in case of sulphanilamide where it reached a maximum of 6.0 mg. per cent.

Of all the six sulpha drugs used for the treatment of infected wounds, sulphathiazole produced the best results, since the discharge was much less, puss cell formation and growth of cocci was arrested on the 8th day. The edges of the wound were clean and soft and ultimate healing took place on the 11th day. Sulphadiazine was found to be the next best.

XXVII. Manufacture of Vitamin A Concentrates

(GANAPATHI, SHIRSAT, BELLARY AND SHAH)

Two known chemical methods of obtaining vitamin A concentrates from shark liver oil were tried for the preparation of large quantities of the concentrate. In the first method, shark liver oil was saponified in an inert atmosphere with potassium hydroxide under standard conditions, the reaction mass was diluted with water and the vitamin A fraction was removed by extraction with ether. The ether extract, after washing, drying and distilling off the solvent, yielded a concentrate of vitamin A, which was free from the disagreeable odour and taste of the original oil. Under the conditions worked out, the recovery of the vitamin was about 80 per cent, and the loss of the ether was about 20 per cent. The method was found to be unsuitable for large scale productions; since it involves the use of large volumes of solvents, and the distillation of ether in large quantities is indeed hazardous. The second method consists in saponifying the shark liver oil with potassium hydroxide and extracting the vitamin from the soap so obtained with ethylene dichloride. The extract, after washing, drying and distilling off the solvent, furnished the vitamin A concentrate free from the disagreeable odour and taste of the original oil. The recovery of the vitamin was the same as in the first method and that of the solvent used, was about 90 per cent. Adopting this method large quantities of vitamin A concentrates are being prepared in the Institute.

Shark liver oil was distilled in a laboratory cyclic, falling film molecular-still, obtained from the Distillation Products, Rochester. The distillation proceeded very smoothly; various fractions were collected at different temperatures and these were assayed for the vitamin A content. The first fraction below 180°C., carried off practically all the disagreeable odour and fractions collected between 180-220°C. contained the bulk of the vitamin A esters. The distillate was pale yellow in colour, free from the disagreeable odour, and could be made pleasant to take by blending with a flavouring oil. The residual oil was free from practically all the bad odour of the original oil and could be taken in without difficulty.

These concentrates could be blended with skimmed milk powder, sugar and flavouring agents and made into 0.5 g. tablets, so that each tablet contained about 2,000 International Units of vitamin A and 200 units of vitamin D. These tablets are nice to take and the vitamin A is not destroyed for the period of 6 months so far tested.

XXVIII. Preparation of 2-Halogenothiazoles

(GANAPATHI AND MRS. VENKATARAMAN)

2-Halogenothiazoles are important intermediates for the synthesis of sulphathiazole and some sulphones. The methods of preparation of 2-chloro or bromothiazole, as described in the literature being far from satisfactory; to effect improvements, 2-aminothiazole was diazotised and the diazo compound decomposed under a variety of conditions. Diazotisation with at least 40 per cent sulphuric acid or with a mixture of phosphoric acid and nitric acid gave the best results, and for the decomposition the best agent found out was a solution of molecular equivalents each of copper sulphate and sodium halide. According to this method, 2-chlorothiazole and 2-bromothiazole were obtained in about 70 per cent yields. A large number of 2-aminothiazole derivatives with different substituents in position 4 and 5 were tried and herein the nature and position of the substituent affected the results. 2-Bromo-5-nitrothiazole which could not be prepared in good yields from 2-amino-5-nitrothiazole by ordinary methods, was prepared by diazotising the aminothiazole derivative in the presence of copper sulphate and sodium chloride.

XXIX. Synthesis of some Sulphones of the Thiazole Group

(GANAPATHI AND MRS. VENKATARAMAN)

By treating about equimolecular quantities of the potassium salt of *p* acetamino-benzenesulphinic acid, in a suitable solvent or by direct fusion, with a number of 2-bromothiazoles differently substituted, a series of sulphones of the thiazoles group have been obtained. Thus 2-bromo-5-nitrothiazole, 2-bromothiazole-5-carboxylate, 2-bromo-5-ethylthiazole, 2-chloro-4 : 5-diethylthiazole and 2-bromo-4-methylthiazole-5-carboxylate yielded the corresponding sulphones. On hydrolysis, the free amino compounds were obtained.

XXX. Structure of Chrysean

(GANAPATHI AND MRS. VENKATARAMAN)

The compound obtained by Wallach by passing hydrogen sulphide into a concentrated solution of sodium cyanide and called "chrysean" has been assigned the structure of 2-thioamido-5-aminothiazole. The preparation of this compound and a proof of its structure were attempted. On repeating the preparation, instead of a compound m.p. 204°C., two products m.p. 222°C. and 146-7°C. were obtained, the mixed m.p. between the latter two being anywhere between 222°C. and 146-7°C. Action of thioformamide on a concentrated solution of sodium cyanide also yielded the product of m.p. 222°C. which tallies with the properties of chrysean. 5-Acetaminothiazole-2-carboxyl amide obtained by degrading this could not be converted by Hoffmann reaction into 5-acetamino-2-aminothiazole, the acetyl derivative of which has been synthesized by an unequivocal method from 2-aminothiazole-5-carboxylate. A number of unsuccessful attempts have been made to synthesize the degradation products of chrysean for identification. 2-Halogenothiazoles react violently with cuprous cyanide but no cyano-compounds could be isolated. 2-Bromothiazole-5-carboxylate could not be converted into 2-bromo-5-acetaminothiazole.

XXXI. Synthesis of Sulphonamidothiazoles

(GANAPATHI, SHIRSAT AND DELIWALA)

In connection with the study on sulphathiazoles, more satisfactory methods than those already available in literature have been worked out for the preparation of 5-substituted 2-aminothiazoles, which constitute the necessary intermediates for the synthesis of 2-sulphanilamido-5-alkyl-thiazoles. The synthesis of the final 2-sulphanilamido-5-alkyl-thiazoles was effected mostly by condensing the appropriate amino-thiazoles with acetyl-sulphanilyl chloride in pyridine medium, followed by hydrolysis of the resulting N⁴-acetylsulphonamides by means of hot dilute hydrochloric acid. The final sulphonamides synthesized are: 5-methyl-, 5-ethyl-, 5-propyl-, 5-isopropyl-, 5-butyl-, 5-amyl-, 5-isoamyl and 5-hexyl-, 2-sulphanilamidothiazoles.

XXXII. Synthesis of Substituted Sulphathiazolones

(RAJAGOPALAN AND DELIWALA)

With a view to explore the antibacterial potentialities of the sulphonamido-thiazolone series, N⁴-butyryl derivatives of 5-methyl-, 5-ethyl-, 5-isopropyl-, 5-amyl-, 5-phenyl-sulphathiazolones have been prepared. In addition, *bis*-(2-N⁴-butyryl and caproyl)-sulphanilyl derivatives of 5, 5'-diethylene-, and 5, 5'-dihexamethylene-2-amino thiazolones have been synthesized. Symmetrical alkylenedi 2-aminothiazolones, hitherto unknown, have been obtained by application of the customary methods for 5-alkyl-2-aminothiazolones, starting with dibasic acids like adipic and sebacic acids.

XXXIII. Synthesis of Sulphanilhydroxamides

(RAJAGOPALAN AND DELI ALA)

A series of sulphonamides derived from N-carbo- and heterocyclic hydroxamides have been synthesized, since a number of N⁴-acylsulphanilhydroxamides have been favourably reported on in preliminary experimental bacterial infections. Among the latter was sulphabenamide or N⁴-caproyl sulphanilhydroxamide, which was said to be effective clinically in streptococcal infections and tuberculosis. This is a new class of sulphonamides involving the preliminary synthesis of some hitherto unknown substituted aryl- and heterocyclic hydroxyl amines. The compounds of this series so far prepared are the N⁴-butyryl sulphanilyl derivatives of β -phenyl-, *s*-3 chlorophenyl-, *s*-4 chlorophenyl-, β -2: 5 dichlorophenyl-, β -3: 4 dichlorophenyl- and β -3: 5 dichlorophenyl hydroxyl amines.

XXXIV. Synthesis in the Sulphone Group

(DELIWALA AND RAJAGOPALAN)

For specific use in tuberculosis and leprosy hydnocarpyl and phthalyl derivatives of sulphanilamide, 4: 4'-diamino-diphenyl-sulphone and 4-nitro-4'-amino diphenyl-sulphone have been synthesized. For the same purpose, a limited number of mono- and di-substituted derivatives of diamino-diphenyl sulphone have also been prepared. A few members of the p-amino phenyl thiazolyl sulphones were also synthesized. These conform to the general formula $H_2N-C_6H_4.SO_2-R$, where R is equal to 4: 5-dimethyl-2-thiazolyl, 5-ethyl-2-thiazolyl, 4-methyl-5-ethyl-2-thiazolyl, 4-methyl-5-propyl-2-thiazolyl, 4-methyl-5-carbethoxy-2-thiazolyl, 5-nitro-2-thiazolyl, and 2-benzimidazolyl methyl groups.

XXXV. Chemotherapy of Experimental Plague

(RAJAGOPALAN, DELIWALA AND HABBU)

Some of the above-mentioned compounds and also of those sulphonamides synthesized prior to the present investigations are undergoing preliminary screening tests as to their efficacy in experimental plague-infected white mice. Particularly promising results have been obtained with sulphadiazine, sulphamerazine, sulphamethazine, and streptomycin. These experiments are now being repeated in greater detail.

XXXVI. Oxidation by Virulent and Avirulent Strains of *Pasteurella pestis*

(S. K. GOKHALE)

Oxidation of certain substances by *P. pestis* using only avirulent strain has been studied before. The object of the present study was to find out whether the enzymatic properties of the bacterial cell are destroyed or get modified when the organism passes from the virulent to the avirulent state. It is known that the antigenic structure of the organism undergoes a change when passing from the virulent to the avirulent state. Experiments on the growth of bacteria have shown no marked change when virulent or avirulent strains have been used. But from such experiments done in a medium containing many substances, it is not possible to get information about the ability of the organism to oxidize different energy sources. For this purpose the study of oxidation of individual substrate was undertaken by employing the manometric technique of Warburg. The 'resting cell' technique of using washed cell suspensions, usually in vogue for such studies was adopted. Oxygen uptakes were determined at 15 minutes' intervals for 2 hours at 27°C. Seven strains of the plague bacillus, three virulent and four avirulent, have been studied. The summary of the results is given below in a tabulated form. It indicates that the cells of the plague bacillus in the virulent and avirulent strains studied have the same enzymatic properties.

TABLE.—Showing the Oxygen uptake in cubic millimetres at the end of one hour per 10 mg. of dry weight of bacteria

Substrate	Average O ₂ uptake in one hour in cubic millimetres	
	Virulent Strains	Avirulent Strains
Glucose	367	376
Galactose	308	323
Maltose	273	260
Lactose	22	24
Sucrose	18	20
dl. Alanine	136	143
l. Cysteine	58	55
Cysteine HCl	38	40
l. Tyrosine	27	27
l. Tryptophane	0	0
Acetate	120	116
Citrate	33	34
d + Tartrate	24	23
Formate	0	0
Butyrate	0	0

XXXVII. Effect of Sulpha Drugs on the Oxidation by *Pasteurella pestis*

(S. K. GOKHALE)

The inhibitory effect of the sulpha drugs on the oxidation by some pathogenic organisms has been the subject of intensive study in recent years; but most of the work is with organisms other than *P. pestis*. The present investigation was undertaken to ascertain the action of some of these drugs on the respiration of the plague bacillus in different substrates and in relation to the stimulating effect of coenzymes like para-aminobenzoic acid. Oxygen uptakes were measured by the Warburg manometric technique of 'resting' washed cell suspensions of the bacteria. The substrate used was 1.0 ml. of 0.05 M glucose and 0.5 ml. of bacterial suspension containing 5 mg. dry weight of bacteria, and different concentrations of the drugs were used. The comparative inhibitory effect of the different drugs is also being studied. The following table gives results obtained with different concentrations of sulphathiazole at 37°C:—

Time in hours	Sulphathiazole concentrations						
	No Sulphathiazole	0.04 M			0.02 M		0.01 M
		O ₂ uptake in ml.	O ₂ uptake in ml.	Percent-age inhibition	O ₂ uptake in ml.	Percent-age inhibition	O ₂ uptake in ml. Percent-age inhibition
1	...	265	48	82	130	51	155 42
2	...	556	69	88	235	58	312 44
3	...	832	75	91	313	63	445 47
4	...	1,078	100	91	370	66	569 47
5	...	1,321	120	91	422	68	678 49

XXXVIII. Treatment of "Tropical Ulcer" with Sulphathiazole Cod-liver Oil Paste

(RADHAKRISHNA RAO AND COLAH)

Early in the year 1944, a research unit from this Institute was sent to East Khandesh to carry out an investigation on cases of chronic ulcer of the skin and underlying tissues, situated mostly on the lower extremities, which were reported to be occurring in large numbers. Extensive laboratory investigations were carried out, and it was found that the ulcer closely resembles "Naga Sore" or "Tropical Ulcer", met in Assam. The incidence of "Tropical Ulcer", has not been reported before in the Bombay Province.

As the treatment of the ulcer has been unsatisfactory, an extensive clinical trial was given to the application of a sulphathiazole paste in water-in-oil emulsion of cod-liver oil prepared at the Institute. Local application of the paste combined with general treatment has yielded very encouraging results.

XXXIX. Preservation of Vitamin C in Sweet Toddy

(RADHAKRISHNA RAO AND MALANDKAR)

Investigations carried out at this Institute during previous years have shown that fresh sweet toddy contains approximately 10 mg. per cent of vitamin C. Further experiments were undertaken, which show that vitamin C in sweet toddy can be preserved by the addition of sodium benzoate, citric acid and sodium metabisulphite, if the toddy is stored in the cold at a temperature of about 4°C. Under these conditions, nearly two-thirds of the original amount of vitamin C was still present in the toddy even after a prolonged storage of 10 months. On the other hand, when it is stored at room temperature in a dark cupboard, addition of the preservatives mentioned above had practically no effect on the preservation of vitamin C. At the end of one month, nearly two-thirds of the vitamin C was destroyed, and at the end of 3 months there was practically no vitamin C left in the toddy.

XL. Normal Prothrombin Time in Indians

(MISS BRAGANCA AND RADHAKRISHNA RAO)

With a view to establish the normal prothrombin level in Indians, and to see if there is any variation as compared with the values reported by the European and American workers, the blood prothrombin level of 115 apparently healthy adults, both male and female, was determined by Quick's method as modified by Fullerton. The thromboplastin used was Russell's Viper Venom, 1 : 10,000 dilution. The crude venom extracted in this Institute was purified by redissolving it in water, centrifuging at high speed to remove the mucus, and drying it by the lyophilic process. The dilute solution prepared from the dry venom could be kept in cold at 4°C. without any change in potency.

Preliminary experiments were also carried out by using thromboplastin, prepared from rabbit's brain by extracting the fresh brain with acetone and drying it. This source of thromboplastin was soon discarded, as it gave a turbid solution which made it difficult to see the first formation of fibrin threads when used for prothrombin determination. The values obtained for the blood prothrombin level of adults examined generally varied from 15 to 25 seconds. These results were found to be in good agreement with those obtained by other workers for normal subjects, and show that there is no variation in prothrombin level of Indians as compared with other nationalities.

Investigations were also carried out on the prothrombin level of blood of patients having positive Wassermann and Kahn tests. Cases are reported by Kark and Lozner, in which positive Wassermann reaction was associated with a prolongation in prothrombin time. Our results showed that there is no variation from the normal in such cases.

XLI. Hypoprothrombinaemia produced by Sulphathiazole in Rats on a Diet free of Vitamin K and cured by synthetic Vitamin K

(MISS BRAGANCA AND RADHAKRISHNA RAO)

Many attempts have been made to utilize common laboratory animals like the rat, guineapig, etc. as experimental animals for the assay of vitamin K. They have resulted only in an irregular production of this deficiency, and have led to the conclusion that mammals are not very susceptible to a purely dietary deficiency of this vitamin.

The observation that bacteria inhabiting the intestines of most mammals are a potent source of supply of vitamin K, has recently led to attempts at producing vitamin K deficiency in rats by partially sterilizing the gut with the sulpha group of drugs. Kornberg, Sebrell, Daft and others have reported that continual administration of sulpha drugs to rats supplemented with a diet deficient in vitamin K can consistently produce hypoprothrombinaemia.

Based on the above observations, preliminary experiments were carried out with a view to producing vitamin K deficiency in the albino rat by the use of sulphathiazole, and subsequent development of an assay-method for vitamin K using the rat, an animal easy to handle, as the experimental animal.

For this purpose, three groups of animals were used. The first group received a synthetic ration which was high in carbohydrates but free from vitamin K and contained sulphathiazole. It was supplemented with 100 mg. of vitamin K per rat per week. The second group of animals received a synthetic ration similar to the first group but without the vitamin K supplement. The third group received the same synthetic ration as the other two groups but without the supplement of vitamin K or the drug.

It was found that within three weeks all the animals of the second group looked emaciated and extremely pale, while some showed bleeding from the nose and ears.

Prothrombin estimations were made by using a modified micro-method of Quick and estimating the prothrombin in the first drop of blood obtained by clipping the tail. It was found that in the majority of the second group of animals, the prothrombin level had fallen to less than 10 per cent, whilst that of the other groups remained normal. These results indicated that the animals receiving vitamin K free diets and the drug had developed severe hypoprothrombinaemia which could be prevented by inclusion of vitamin K. The animals of the third group continued taking the synthetic diet for over two months, but even then failed to develop a deficiency of vitamin K as shown by the prothrombin level. This confirms the findings of other workers who reported that a purely dietary vitamin K deficiency could not easily be produced in the rat.

With a view to see whether vitamin K could cure the severe hypoprothrombinaemia produced in the rat by feeding sulphathiazole, another set of animals was started and kept on the same diet as the second group. At the end of three weeks when the animals had developed severe hypoprothrombinaemia, they were given a supplement of vitamin K and prothrombin estimated at the end of 48 hours. It was found that in every case the prothrombin returned to the normal level.

Experiments are now in progress to determine the minimum dose of vitamin K required to bring back to normal the prothrombin level of animals suffering from severe prothrombin deficiency produced as a result of feeding the sulphadiazole drug and vitamin K deficient diet.

XLII. Histopathological Changes in Hypoprothrombinaemia produced by Sulphathiazole in Rats on a Diet free of Vitamin K

(MISS BRAGANCA AND RADHAKRISHNA RAO)

In order to study the effect of continued feeding of Sulphathiazole together with a vitamin K free diet on the liver and other organs, animals in the different groups were killed at various intervals. Post-mortem examination of the animals, showing marked hypoprothrombinaemia, revealed that in the majority of cases the liver and the kidneys were enlarged and pale. Haemorrhages were found in various parts of the body, the most common site being the urinary bladder, the epididymis, testicles and the pelvic region.

In the case of animals, receiving the drug together with a vitamin K free diet, it was found that till the end of second week there was practically no change in the histology of the liver. During the third week, however, marked prolongation of prothrombin time was generally associated with considerable fatty change in the liver. Necrosis of the parenchyma around the central veins was evident in some cases. Animals of this group were not kept for more than 24 days. In few cases the kidneys were also examined, and stained sections showed slight cloudy swelling of the epithelium of the convoluted tubules.

The livers of animals in the group receiving vitamin K and the drug showed practically normal histological appearances, even though these animals had been receiving the drug for periods varying from 60 to 72 days. The kidneys, however, showed cloudy swelling of the tubular epithelium similar to that seen in the above group.

It is thus evident that the addition of vitamin K to a synthetic vitamin K free diet to which sulphathiazole has been added, prevents the fatty change in the liver. On the other hand, histological changes in the kidneys could not be prevented by the addition of vitamin K.

XLIII. Experimental Hepatic Cirrhosis

(RADHAKRISHNA RAO, DATTA AND KRISHNAN)

Recent researches have shown that necrosis of the hepatic parenchyma with occasional periportal fibrosis could be produced in experimental animals by feeding deficient diets. Clinical experience has similarly shown that decompensated portal cirrhosis in India is mostly a disease of the poor in whom dietary deficiencies are very common. Based on the above observations, the following experiments were devised to study the possible role of nutritional factors in the production of cirrhosis of the liver.

Young healthy albino rats were fed on purified synthetic diets low in protein and devoid of the factors of the vitamin B₂ complex, and the resulting chemical and histological changes in the liver were studied. It was observed that when

the level of protein (purified casein) was supplied at 5 per cent level in the basal diet devoid of vitamin B₂ complex, the animals failed to grow, and did not survive for more than 2 or 3 months. They developed fatty livers. When the protein level was increased from 5 per cent to 8, 10 and 18 per cent in the different diets, there was better growth and the average duration of life was prolonged. Chemical analysis of the liver, however, showed accumulation of fat. The livers were enlarged and their weight was out of proportion to the body weight. On the other hand, control animals in the different groups which received a supplement of 0.5 g. of dried brewer's yeast per rat per day, to provide the different vitamins of the B₂ group, grew well and were quite healthy even after long periods on the experimental diets. In general weight of the liver was proportional to the body weight and the organ was normal in appearance. Liver fat was normal and fairly constant in amount, except in the 5 per cent casein group which showed slight increase. A detailed account of the experiments is given below.

Effect of various levels of dietary protein (Casein) on the histological and biochemical changes in the liver of rats.—Young healthy albino rats with initial body weights varying from 35 to 50 g. were divided into 4 groups, each of 12 rats. They were fed on purified synthetic diets in which the percentage of protein (casein) was 18, 10, 8 and 5. Each rat was given in addition, 2 drops of cod-liver oil and 0.5 g. of dried brewer's yeast. The rats were fed *ad libitum* and they were maintained on these diets for long periods at the end of which they were killed at intervals varying from 230 to 416 days.

The animals in all the groups looked quite healthy and there was no sign of any deficiency symptoms. On 5 per cent casein diet, however, the growth of the rats was much inferior to other groups, and the average liver weight of the animals in this group was slightly higher in proportion to their body weight.

The livers of rats from the different groups were analyzed for their moisture, fat, protein and glycogen content. It was found that when the values were calculated in the terms of gram per 100 grams of body weight, no significant differences in the amount of water, fat, protein and glycogen in the livers were noticed in different groups except in the case of the 5 per cent casein group which showed slight increase in the water and fat content.

Histological examination of the livers showed that in the 18 per cent casein group, fatty change was completely absent, whereas in the 10, 8 and 5 per cent casein groups it tended to increase, as the protein level in the diet was reduced. Necrosis of the parenchymal cells of the liver was not a prominent feature and was only present in the very low protein groups. Occasional patchy fibrosis was found in the 10 and 5 per cent groups.

Effect of the deficiency of the vitamins of B₂ complex at various levels of dietary protein (casein) on the histological and biochemical changes in the liver of rats.—A total number of 108 young albino rats were used in this series of experiments. The composition of the basal diets which contained varying proportion of casein, namely 18, 10, 8 and 5 per cent was similar to that used in the previous series of experiments. Each rat in this series received 2 drops of cod-liver oil and only 20 mg. of thiamine per day but no yeast. It was observed that the growth and survival period of rats in these groups were proportional to the level of casein in the diet. Animals

receiving 5 per cent casein failed to grow and did not survive for more than 2 or 3 months. Symptoms suggestive of vitamin deficiencies were noticed in many animals, especially those receiving low protein diets.

In general the liver weight of the animals was inversely proportional to the level of casein in the diet, i.e. as the percentage of casein in the diet was decreased, the average weight of the livers per 100 g. of body weight was increased. In animals receiving 5 per cent casein in their diets, the results, however, were not consistent. With the exception of a few rats which showed liver weight of about 10 to 11 g. per 100 g. of body weight (normal range 3.8 to 4.5 g. per 100 g. of body weight), the average liver weight in this group did not show the same relationship to body weight as that observed in the other groups.

The estimation of water, fat and protein contents of the liver showed that this increase in the weight of the liver in the low protein groups was not due to an increase in any single constituent. The amounts of water, fat and protein in liver showed an increase which was also inversely proportional to the level of casein in the diet, i.e. when the percentage of casein in the diet was low, the liver on an average contained more water, fat and protein than the liver of animals that received higher levels of casein in the diet.

However, as mentioned above, at the lowest intake of protein, i.e. at 5 per cent level of casein, the increase both in the liver weight and in the amounts of water, fat and protein in the liver was not similarly proportional. This is mainly due to the low food intake, consequent gradual fall in body weight and the short span of life when the level of protein in the basal diet was reduced to 5 per cent.

Histological examination of the liver of the animals in the various groups corroborated the chemical findings. Varying degree of fatty change in the liver was present in the different groups. The extent of fatty change in the liver was inversely proportional to the level of protein in the diet, and was higher in the 8 and 10 per cent groups than in the 18 per cent group. In the 5 per cent protein group, however, it was found to be much lower than in any of the other groups. Necrosis of the parenchymal cells of the liver appeared to be on the increase as the level of protein in the diet was reduced from 18 to 5 per cent. Occasional patchy fibrosis was found in the groups receiving 10 and 8 per cent level of protein in their diets. In the 18 per cent protein group it was altogether absent.

Effect of restricted intake of protein in an otherwise adequate diet on the biochemical and histological changes in the liver of rats.—In the previous experiments the different diets were given to rats *ad libitum*. It was found that due to variation in the total amount of food consumed, there was considerable difference in the intake of protein in the same group of animals. It was, therefore, considered desirable to limit the intake of protein at particular levels. Accordingly 4 groups of rats were given purified synthetic diets in such quantities that rats from the first group received a maximum of 1.2 g. of casein per rat per day, while those in the second, third and fourth groups received 0.6, 0.4 and 0.2 g. of casein per rat per day. Each rat was given in addition 0.5 g. of yeast per day. This series of experiments was continued for 150 days after which one animal from each group was killed at regular intervals,

A record of the daily food intake of the animals as also their weekly body weight was kept. It was found that the growth of the animals in this group was proportional to the intake of protein. In addition to the chemical and histological examination of the liver, the total plasma protein was also estimated. These investigations are in progress.

XLIV. Immunizing Value of Rabies Vaccine

(R. S. RAO)

A beginning had been made in 1942 to test the immunizing potency of rabies vaccine prepared in this Institute by Habel's modifications of Webster's technique. The Haffkine Institute inbred strain of white mice, 17 g. in weight, has been found to be eminently satisfactory for this purpose. As it is not practicable to test every batch of vaccine, periodical examination of a batch picked at random, affords an efficient check on the efficacy of the vaccine.

The number of "M.L.D. protection" as determined by the 50 per cent end point method has been found to vary with different batches from about 30,000 to 100,000.

XLV. Comparative immunizing Values of chloroformed and phenolized Rabies Vaccines

(R. S. RAO)

Webster in U.S.A. has advocated the use of chloroformed vaccines in preference to phenolized vaccines. Testing several samples obtained from commercial firms, he found that chloroformed vaccine afforded considerably more protection than phenolized vaccine. An experiment was conducted to assess the relative merits of the two vaccines. Chloroformed vaccine was prepared by making a 5 per cent suspension of rabbit brain virus in 2 per cent chloroform-saline. A 5 per cent phenolized vaccine was also prepared from a portion of the rabbit's brain used for the chloroform vaccine. The result of the test showed that "M.L.D. protection" for the chloroformed and phenolized vaccine was 63,100 and 35,480 respectively, which indicates only a slight superiority of the chloroformed vaccine over the phenolized vaccine.

LIST OF PAPERS PUBLISHED FROM THE HAFFKINE INSTITUTE
DURING THE YEARS 1944, 1945 AND 1946

1. BRAGANCA, B. M. (MISS) & RAO, M. V. R. (1946). Production of Hypoprothrombinaemia in the Rat by feeding Sulphathiazole and its Cure with synthetic Vitamin K. *Cur. Sci.* **15**, pp. 126-127.
2. BRAGANCA, B. M. (MISS) & RAO, M. V. R. (1946). Prothrombin Time in Normal Indians. *Ind. Med. Gaz.* **81**, pp. 244-246.
3. BRAGANCA, B. M. (MISS) & RAO, M. V. R. Hypoprothrombinaemia produced by Sulphathiazole in Rats on a Diet free of Vitamin K and cured by synthetic Vitamin K. (In press).
4. DIKSHIT, B. B. & GARDHAM, A. J. (1945). Control of Sepsis in War Wounds by Pre-Surgical Treatment. *Ind. Med. Gaz.* **80**, pp. 121-125.
5. DUTTA, N. C. (1945). Minimum Requirement of Fat in Human Nutrition. *Sci. Culture* **10**, pp. 390-394.
6. DUTTA, N. C. (1945). Influence of Different Fats and Oils on the Composition of Body Fat and the Lipoid Content of the Liver of Rats. *Ann. Biochem. Exper. Med.* **5**, pp. 109-116.
7. GANAPATHI, K. (1945). Penicillin. *Ind. Journ. Pharm.* **7**, pp. 93-103.
8. GANAPATHI, K. & VENKATARAMAN, A. (MRS.). (1945). Chemotherapy of Bacterial Infections. Part XI. Synthesis of some Derivatives of Diphenylsulphone. *Proc. Ind. Acad. S. i. (A)* **21**, pp. 34-40.
9. GANAPATHI, K. & VENKATARAMAN, A. (MRS.). (1945). Chemistry of the Thiazoles. Part I. Synthesis of 5-Aminothiazole Derivatives, Part II. Synthesis of 4-Aminothiazole Derivatives, Part III. Synthesis of Thiazole Derivatives Unsubstituted in Position 2: An Evaluation of Various Possible Methods. *Proc. Ind. Acad. Sci. (A)* **22**, pp. 343-378.
10. GOKHALE, S. K. & INDERJIT SINGH. (1945). The Effect of Ammonium on the Potassium Content of Unstriated Muscle and its Relation to the Contraction produced on withdrawal of Chemical Substances from around the Muscle. *Proc. Ind. Acad. Sci. (B)* **21**, pp. 202-207.
11. HAZRA, A. K., LAHIRI, D. C. & SOKHEY, S. S. (1945). A New Anti-Snake-Venom Serum. *Cur. Sci.* **14**, pp. 20-21.
12. HAZRA, A. K., LAHIRI, D. C. & SOKHEY, S. S. (1946). On the Standardisation of Haffkine Institute Polyvalent Anti-Snake-Venom Serum against the Venoms of the four Common Indian Snakes (Cobra, Common Krait, Russell's Viper and Saw-scaled Viper). *Bull. Hlth. Organis. Leag. Nations* **12**, pp. 384-389.
13. JOSHI, R. A. (1944). Investigation on Undulant Fever in the City of Bombay. *Ind. Med. Gaz.* **79**, pp. 369-372.

14. LAHIRI, D. C. (1946). Antitoxins and Sera. Study of their Nature, Production and Assay. *Ind. Journ. Pharm.* 8, pp. 150-168.
15. LAHIRI, D. C. A New Anti-Allergic Serum. (In press).
16. LAHIRI, D. C. A Method of Improving the Solubility of dehydrated Concentrated Horse-Serum Immune Globulins by Addition of Bile Salt and Extraction with Ether. (In press).
17. MIRDAMADI, H. & DE, S. P. (1946). A Modification of Ramon's Flocculation Method. *Cur. Sci.* 15, p. 314.
18. MIRDAMADI, H. & DE, S. P. Use of Tolu Balsam in Ramon's Flocculation Reaction. (In press).
19. PATEL, B. V. (1944). Thiazole Derivatives of Sulphanilamide in Monkey Malaria. *Quart. Jour. Pharm. Pharmacol.* 17, pp. 297-301.
20. PATEL, B. V. Local Treatment of Infected Experimental Wounds, (In press).
21. RAJAGOPALAN, S. (1944). Bacterial Chemotherapy. IV. Synthesis of N⁴, N¹-Diacyl Sulphanilamides, V. Synthesis of Phenolic Azo-dyes derived from the Sulphonamides. *Proc. Ind. Acad. Sci. (A)* 19, pp. 1-14.
22. RAO, M. V. R., COLAH, R. B. M. & KALLE, R. A. (1945). Tropical Ulcer, in East Khandesh. Treatment with Sulphazole cod-liver oil Paste. *Ind. Med. Gaz.* 80, pp. 128-130.
23. SALVEKAR, P. M. (1945). Minimum Size of the Sample Required for Experimental Work to estimate the Population Mean with a Specified Degree of Accuracy for a Specified Level of Reliability. *Jour. Univ. Bombay* 13, pp. 2-6.
24. SHARIF, M. (1945). On the Structure of the So-Called 'Penis' of the Oriental Cat-flea *Ctenocephalides felis* Subsp. *orientis* (Jordan), and Homologies of the External Male Genitalia in Siphonaptera. *Proc. Nat. Inst. Sci. India* 11, pp. 80-95.
25. SHARIF, M. Effects of Constant Temperature and Humidity on the Development of the Larvae and the Pupae of the three Indian Species of *Xenopsylla* (Siphonaptera). [In press].
26. SHARIF, M. Nutritional Requirements of Flea Larvae, and their Bearing on the specific Distribution and Host Preference of the three Indian Species of *Xenopsylla* (Siphonaptera). [In press].
27. SHARIF, M. The Water Relationship of the Larva of *Xenopsylla cheopis* (Siphonaptera). [In press].
28. SOKHEY, S. S. & HABBU, M. K. (1944). A Broth Cholera Vaccine. *Cur. Sci.* 13, p. 230.
29. SOKHEY, S. S. & HABBU, M. K. Casein Hydrolysate Direct Cholera Vaccine. (In press).

30. SOKHEY, S. S. & HABBU, M. K. Biological Assay of Cholera Vaccine. (In press).
31. SOKHEY, S. S. & HABBU, M. K. Rate of Growth of *Pasteurella pestis*. (In press).
32. SOKHEY, S. S. & WAGLE, P. M. (1946). A Note on the Use of Sulphonamides in the Treatment of Plague in the Field. *Ind. Med. Gaz.* 81, pp. 343-346.
33. SOMAN, D. W. (1944). Observations on a 24-Hour Rat Test (Aschheim-Zondek Modification) for the Diagnosis of Pregnancy. *Ind. Med. Gaz.* 79, pp. 68-70.
34. SOMAN, D. W. (1944). The Value of D. E. C. Medium in the Isolation of Intestinal Pathogens. *Ind. Med. Gaz.* 79, pp. 479-482.
35. SOMAN, D. W. (1945). The Distribution of Typhoid Vi Agglutinins in Normal Sera with special reference to their Diagnostic Value in Typhoid Fever. *Ind. Med. Gaz.* 80, pp. 90-93.
36. SOMAN, D. W. (1945). Malaria in Pandharpur. *Jour. Malar. Inst. India* 6, p. 99.
37. SOMAN, D. W. (1946). A Comparative Study of Saponin Broth as a Blood-Culture Medium for Isolation of Enteric Group of Organisms. *Ind. Med. Gaz.* 81, pp. 121-122.
38. SOMAN, D. W. (1946). A Preliminary Study of Six-Hour Rat Test for the Diagnosis of Pregnancy. *Ind. Med. Gaz.* 81, pp. 134-136.
39. SOMAN, D. W. Paul-Bunnell Test in the Diagnosis of Glandular Fever with Report on seven cases. (In press).
40. SOMAN, D. W. & NAIL, S. K. (1945). Sub-Types of Cholera Vibrio Isolated from Cholera Patients in Bombay. *Ind. Med. Gaz.* 80, pp. 512-514.
41. WAGLE, P. M. (1944). Sulphadiazine in the Treatment of Bubonic Plague. *Ind. Med. Gaz.* 79, pp. 585-589.
42. WAGLE, P. M. & COLAH, R. B. M. Prognostic Significance of Leucocytic Count in Bubonic Plague. (In press).

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S. S. SOKHEY, Kt., Maj.-Gen., I.M.S.,
Director, Haffkine Institute.

