



सत्यमेव जयते

REPORT
OF
THE HAFFKINE INSTITUTE

For the Year 1954

BY

Dr. P. M. Wagle, M.D., D.P.H.
Director, Haffkine Institute, Bombay

BOMBAY
PRINTED AT THE GOVERNMENT CENTRAL PRESS

Obtainable from the Government Publications Sales Depot, Institute of Science Building, Fort, Bombay (for purchasers in Bombay City); from the Government Book Depot, Charni Road Gardens, Bombay 4 (for orders from the mofussil) or through the High Commissioner for India, India House, Aldwych, London, W.C.2 or through any recognized Bookseller.

1955

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1954

CONTENTS

PART I

GENERAL

	Page.
I. Staff	1
II. General Review	2-4
III. Department of Vaccines	4-5
A. <i>Plague Vaccine</i> ,	5-6
B. <i>Cholera Vaccine</i>	6
C. <i>T. A. B. Vaccine</i>	7
D. <i>Media Section</i>	7-8
E. <i>Section of Public Health Chemistry and Bacteriology</i>	8-9
IV. Department of Antitoxins and Sera	9-12
V. Department of Entomology	12-13
A. <i>Snakes</i>	13
B. <i>Examination of rats from Bombay City and Port Trust Area.</i>	14
C. <i>Examination of rats from steamers and other sources</i>	14-20
VI. Department of Clinical Pathology and Diagnostic Reagents	21
A. <i>General Bacteriological Work</i>	21-23
B. <i>Diagnostic Reagents</i>	23-24
C. <i>Histopathology Section</i>	24-26
D. <i>Special Investigations</i>	27
E. <i>Training and Research Scholars</i>	27
VII. Department of Pharmacology	27-32
VIII. Department of Chemotherapy	32-34
IX. Department of Biochemistry	34-35
X. Department of Virus Diseases including Rabies	35-40
XI. Blood Bank Department	40-41
XII. Training of Health Personnel	41-42
XIII. Critics Club	42
XIV. Miscellaneous	43-44
XV. Income and Expenditure	44

CONTENTS

PART II

RESEARCH WORK

	PAGE.
I. Antigenic Structure of Plague Bacillus and its Relation to Immunity.	44-45
II. Eijkman's Test	46
III. Preliminary Studies on Immunity Response in mice with mixed Antigens (Bacterial Vaccines and Toxoids).	47
IV. Purification of Antitoxin by Enzyme Digestion Treatment ...	47-48
V. Activity of Different Fractions of Rabbit and Horse Antivenin Sera ...	48
VI. Proteases in Cobra and Russell's Viper Venoms	48
VII. Common Antigens in Pasteurella	48
VIII. Extraction of Antigens of <i>P. pestis</i> using Ethylene Glycol and Diethylene Glycol.	49
IX. Sex Ratio and Specificity of Some Fleas on Bombay Rats ...	49-50
X. Venom Tolerance in Russell's Viper	50
XI. The Influence of Snake Venom and Sera on Rabbit Erythrocytes ...	51
XII. Ratex as a Rodenticide	51-52
XIII. Immunity Response to Semple's Antirabic Vaccine	52-53
XIV. A Lyophilised Antirabic Vaccine	53-54
XV. A Lyophilised Canine Vaccine	54-55
XVI. Chloromycetin in Typhoid Fever	55
XVII. Pregnancy Test	55
XVIII. Anaerobically Grown Typhoid Organisms in Widal Test ...	55
XIX. Tropical Eosinophilia	56
XX. Use of Dyes in Serodiagnostic Tests for Syphilis	56
XXI. Studies on Cardiolipin-Type of Phospholipids and their use in the Serodiagnosis of Syphilis.	57
XXII. Studies on Influenza Virus	57-58
XXIII. Further Studies on Laboratory Diagnosis of Smallpox	59-60
XXIV. Further Observations on the Biological Test for the Diagnosis of Rabies.	60
XXV. Studies on Complement Fixation Test in the Diagnosis of Rabies ...	60-61
XXVI. Potency Test on Hyperimmune Rabies Serum... ..	61
XXVII. Studies on Street Virus of Rabies	61-62
XXVIII. Studies on Avianized Rabies Vaccine (Flurry Strain)	62
XXIX. Experimental Cholera in Rabbits	62
XXX. Investigation on the Sedative and Hypotensive Principles in Rauwolfia Serpentina, Benth.	62-63
XXXI. Detection and Determination of Saccharin in Tea	63
XXXII. Antigenicity Tests for Protein Hydrolysate	64

CONTENTS

iii

	PAGE.
XXXIII. Study on the Stability of Vitamins in Oral Preparations. Part III : Effect of Crude Liver Extract, Alkaloids and Enzymes on Vitamin B ₁ .	64
XXXIV. Indigenous Drugs in Experimental Diabetes	64-65
XXXV. Studies in the Synthesis of Vitamin B ₁ Part I : Use of Acrylonitrile as an Intermediate for the Synthesis of the Pyrimidine Moiety of Thiamin.	65
XXXVI. Studies in the Synthesis of Vitamin B ₁ Part II : Use of Lithium-Aluminium Hydride in the Synthesis of Pyrimidine Part of Thiamin.	65-66
XXXVII. Studies in the Synthesis of Vitamin B ₁ Part III : Use of Lithium-Aluminium Hydride in the Synthesis of Thiazole Moiety of Thiamin.	66
XXXVIII. Chemotherapy of Bacterial Infections : Synthesis of N ¹ -Benzimidazolyl Sulphanilamides and Metanilamides.	66-67
XXXIX. Study of Serum Protein Fractions in Cirrhosis of Liver	67-68
XL. Preparation of Highly Potent Anti-human Rabbit Serum	68
XLI. Haemopoetic Response following Blood Donation in Professional Blood Donors.	68
XLII. Amino Acid Composition of Human Milk	69
XLIII. Influence of Fermented Food-stuffs on Fatty Infiltration of the Liver in Rats.	69-71
XLIV. Experimental Hepatic Necrosis	71-72
List of Papers Published from the Haffkine Institute during the year 1954.	73-74

**REPORT OF THE HAFFKINE INSTITUTE FOR
THE YEAR 1954**

PART I

GENERAL

I. Staff

DIRECTOR

Dr. P. M. Wagle, M.D. (Bom.), D.Bact. (Manch.), D.P.H. (Lond.),
D.T.M. (Liverpool).

ASSISTANT DIRECTORS

Dr. D. W. Soman, M.D. (Bom.), B.Hy. (Bom.), D.P.H. (R.C.P.S.),
D.T.M. & H. (Lond.), B.M.S. (I), Assistant Director I/c Department of
Virus Diseases including Rabies.

Lt. Col. J. R. Dogra, M.D., Ch.B. (Edin.), D.T.M. (Liverpool), Assistant Director
I/c Department of Clinical Pathology up to 4th August 1954 (F.N.).

Dr. B. G. Modi, M.B., B.S. (Bom.), F.C.P.S., D.P.B., B.M.S. (II), Officiating as
Assistant Director I/c Department of Clinical Pathology from 4th August
1954 (F.N.).

Dr. N. K. Dutta, M.B. (Cal.), D.Phil. (Oxon), B.M.S. (I), Assistant Director
I/c Department of Pharmacology.

Dr. P. J. Deoras, B.Sc., M.Sc. (Nagpur), Ph.D. (Durham), Assistant Director
I/c Department of Entomology.

Dr. A. K. Hazra, M.B., B.S. (Bom.), D.T.M. (Cal.), B.M.S. (I), Assistant
Director I/c Department of Antitoxins and Sera.

Dr. C. V. Deliwala, M.Sc., Ph.D., Officiating as Assistant Director I/c Depart-
ment of Chemotherapy.

II. General Review.

In general the activities of the Institute remained the same as in previous years. Production of large quantities of prophylactic vaccines, *viz.*, plague, cholera, typhoid and anti-rabic vaccine, prophylactic and curative antitoxins and toxoids, sulpha drugs and vitamin tablets, parenteral solutions and diagnostic work for hospitals and practitioners claimed its resources in funds and personnel. It also served as an analytical laboratory for enforcement of Drugs Act, 1940, for the Bombay State and other States in Indian Union and as a provincial bacteriological laboratory for the Bombay State. The fact that the demand for the biological products made available by it is, on the whole, steadily on the increase should be taken as a measure of confidence the public has learnt to place on its efficacy and guarantee of their purity. Despite the pressure of routine work most of the senior members of the staff carried out research work detailed elsewhere in the report. Some of the research problems bearing on the various activities of the Institute were also carried out with the generous assistance of the State Government, Indian Council of Medical Research and under Fellowships granted by the Unichem Laboratories and the Reptakos Board of Medical Research, Bombay.

The sales amounted to Rupees twenty-seven lakhs while the expenditure was twenty-two lakhs, during the financial year 1953-54.

The demand for the plague vaccine was low as in the previous year, *viz.*, one million millilitres—an indication that the country is kept comparatively free from plague infection as compared to previous years. The demand for cholera vaccine was the same as in 1952, *viz.*, 3-8 million millilitres which is considerably lower than the demand for it in 1953, when about twenty million millilitres were supplied owing to wide-spread infection all over the country. There is thus indication that incidence of plague and cholera infection in the country is falling as a result of improved sanitary control measures.

Although the demand for parenteral solutions was lower than in the previous year, the demand for these products appears to be steadily on increase year by year. Seventy-two thousand bottles were issued this year as compared to sixty-eight thousand in 1952 and ten thousand bottles in 1947.

One of the bottlenecks in the advancement of cholera research has been removed by the discovery of a suitable laboratory animal for inducing experimental cholera infection. It has been found that suckling rabbits, ten days old, are eminently suitable for this purpose and the infection resulting in them resembles human cholera infection. As a result of this excellent work it will be possible in future to test many drugs for their prophylactic and curative value with a degree of accuracy hitherto unknown in cholera research.

Work has been undertaken to determine the part the issue and humoral immunity play in building resistance against plague infection. The work has not yet progressed far enough to draw any conclusions.

The demands for antitoxins and sera have continued to be on increase both from different States in Indian Union as well as from the Armed Forces. Demands also came from foreign countries. Production was further increased to meet the increasing demand and over Rupees eight lakh seventy thousand worth of sera were issued to different parties during the year. The question of further expansion of antitoxins and toxoid production has been taken up with Government.

Further work on the composition of Russell's viper venoms have brought out the fact that the blood coagulating activity of the Russell's viper venom is due to the presence of powerful proteolytic enzymes. Attempt is being made to isolate these enzymes. Proteolytic enzymes are also found to be present in Cobra venom, but their action is comparatively less powerful.

As the Diphtheria toxoid "NAFT" was not found to be more potent than some of the other known Diphtheria prophylactics, it has been decided not to undertake routine manufacture of this product.

In view of the proposed manufacture of Triple Vaccine against Tetanus, Diphtheria and Whooping Cough, investigation was undertaken to study the effect of administration of combined antigens on their immunity responses by using TAB Vaccine and tetanus toxoid as a prophylactic. Results so far obtained have not suggested diminution of immunity response to multiple antigens.

The pressure of work in the Department of Pharmacology continued to be very heavy, there being 20 per cent. increase in the number of samples tested. For the first time a large number of samples of spirituous medicinal preparations was sent by Prohibition Department for analysis. Certain manufacturers have put on the market pharmacopoeial tinctures which, on examination, were found to be nothing but coloured alcohol. Out of 2,361 samples analysed 36 per cent. were found to be of non-standard quality which shows an increase of about 9 per cent. over the figure of previous year. Under the Drugs Act, 1453 samples were examined and of these 633 were found to be of non-standard quality, the percentage being 44 as compared to 35 per cent. last year.

As the methods available for the determination of saccharin present in prepared tea were found to be unreliable, a satisfactory method for its detection was worked out. Reserpine, the hypotensive and sedative principle present in *Rauwolfia Serpentina Benth* has been isolated in good quantities from the 'oleoresin' fraction of the alcoholic extract of the root. A simple and economical method has been worked out for the manufacture of reserpine.

The work on the assessment of the pulicidal power of pyrethrum was continued further by the Department of Entomology. The powder formulations which are found to be most effective in the laboratory experiments will now be manufactured on larger scale to undertake field experiments. The experiments on the rearing of snakes in the snake-farm have also been continued. Conditions for keeping the Cobras and Russell's vipers in the farm have now been fully worked out and studies are being continued to observe the conditions necessary for rearing Kraits and Echis. The latter snake appears to be more fastidious about its requirements. Success has not yet been attained in breeding any of the snakes in the farm. As mentioned in the last report, the yield of the venom is more and longevity of the snakes greater, on the whole, in the case of snakes kept in the farm as compared to those kept in the cages.

The demand for anti-rabic vaccine is increasing year by year. 9,451 persons attended for treatment as compared to 8,714 in 1953, and 7,779 in 1952. One case of hydrophobia was reported from amongst those who were treated at a mofussil centre, thus indicating the vaccine alone is not the last word in the prophylaxis of rabies. The anti-rabic serum which was prepared on

an experimental basis and was found to be potent on test in the laboratory has now been issued to certain selected anti-rabic centres in the State for trial in so-called 'bad' cases in conjunction with vaccine. In the meanwhile, production of more serum for experimental purposes will be continued.

Experiments with avianized rabies vaccine have not yet given hopeful results. Work is being continued, particularly with a view to see, what role lyophilisation plays in the loss of potency of this vaccine. With regard to the standardisation of laboratory methods for diagnosis of small-pox, a complement fixation test has been worked out which would enable a quicker diagnosis to be made in suspected cases of small-pox.

The Department of Chemotherapy continued to manufacture sulphathiazole powder, tablets and injectibles, vitamin tablets of different composition, and sulphatrone injectibles. The sale of sulphathiazole tablets was less this year than in the previous year, but the sale of vitamin tablets to some extent greater. About 20,00,000 sulphathiazole tablets and ten million vitamin tablets were sold to government institutions and hospitals, private medical practitioners and charitable institutions. The slightly higher sale in case of vitamin tablets is a result of the introduction of Health Insurance Scheme in Bombay City during the last quarter of the year under review. Consequent on the introduction of the Health Insurance Scheme, the Department also undertook tableting of quinine, sulphadiazine and sulphaguanidine powders for supply to workers under the Scheme. In addition, distribution of quinine and its substitutes which was formerly being done by the Superintendent of Prohibition and Excise was also entrusted to the Department this year. In addition to this routine work, investigations were continued into synthesis of the pyrimidine and thiazole moieties of Thiamine, and in the chemistry of thiazoles with particular reference to compounds which might possess possible therapeutic value.

The Department of Clinical Pathology gave diagnostic aid to various government and municipal hospitals and to some charitable hospitals in the City and free diagnostic aid was given to private medical practitioners in case of infectious diseases. Over 24,000 samples were examined during the year. A Unit was sent to Kaira to make a survey of hook-work infection in the district. The survey work is expected to be completed in the beginning of April 1955. Besides doing diagnostic work for the hospitals, the department also served as a reference laboratory for venereal diseases by production of standard reagents and checking of their results. The Blood Bank Department supplied as usual dried plasma to hospitals and private practitioners in the City and in the mofussil.

Besides the research work done by members of the staff, facilities were provided to young science graduates to do research work for their post-graduate degrees. Seven students worked in the Institute for their post-graduate degrees during the year.

III. Department of Vaccines.

During the year under review the demands for plague vaccine were practically the same as of last year, while the demands for cholera vaccine were much less than that of the last year. The demands for T. A. B. Vaccine were slightly higher than last year.

1.1 million millilitres of plague vaccine, 3.8 million millilitres of cholera vaccine and 3,90,082 millilitres of T. A. B. Vaccine were issued as against 1.1 million millilitres of plague, 19.8 million millilitres of cholera and 3,21,225 millilitres of T. A. B. Vaccine during the year under review.

The fall in demand for cholera vaccine is due to the unprecedented outbreak of cholera all over the country during the year 1953-54 and consequent mass immunization of the population undertaken to combat it in that year.

The total quantity of vaccines issued during the year 1954-55 is 53,97,605 millilitres.

The demands for the vaccines were received from different States of the country and for plague vaccine from some foreign countries also.

The state-wise distribution of plague vaccine is given below :—

A. PLAGUE VACCINE.

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

<i>Months.</i>					<i>Quantity in millilitres.</i>
January	..	..	..	..	4,93,120
February	..	..	..	..	14,970
March	..	..	..	..	2,300
April	..	..	..	..	37,632
May	..	..	..	..	44,320
June	..	..	..	..	2,850
July	..	..	..	..	8,534
August	..	..	..	..	64,440
September	..	..	..	..	1,17,300
October	..	..	..	..	1,45,767
November	..	..	..	..	8,756
December	..	..	..	..	2,08,360
Total ..					11,48,349

Issues of plague vaccine from the Institute since its beginning in quinquennial periods are as given below :—

<i>Year.</i>					<i>Quantity in millilitres.</i>
1898-1901	..	..	..	..	8,601,123
1902-1906	..	..	..	..	10,366,611
1907-1911	..	..	..	..	10,752,786
1912-1916	..	..	..	..	13,702,098
1917-1921	..	..	..	..	20,003,682
1922-1926	..	..	..	..	21,607,908
1927-1931	..	..	..	..	17,787,729
1932-1936	..	..	..	..	19,909,176
1937-1941	..	..	..	..	20,078,811
1942-1946	..	..	..	..	44,528,208
1947-1951	..	..	..	..	65,757,991
1952-1954	..	..	..	..	7,077,856
Total ..					260,173,979

The Plague Vaccine was issued to India and other countries from this Institute. The demands within and outside India met during the year under report are shown below :—

<i>India.</i>				<i>Quantities in millilitres.</i>	
Bombay	...	...	...	1,08,534	
Bihar	...	...	...	61,290	
U. P.	...	...	...	56,520	
M. P.	...	...	...	1,31,200	
Hyderabad	...	...	...	1,050	
Delhi	...	...	...	200	
Vindhya Pradesh	...	...	...	20,050	
Madhya Bharat	...	...	...	1,000	
West Bental	...	...	...	1,00,060	
Travancore-Cochin	...	...	...	4,000	
Madras	...	...	...	2,06,075	
Rajasthan	...	...	...	3,020	
Saurashtra	...	...	...	480	
East Punjab	...	...	...	2,000	
Defence Services	...	...	...	2,795	
Total				6,98,274	6,98,274

<i>Foreign Countries.</i>					
Burma	...	...	...	4,50,000	
Malaya	...	...	...	60	
Sikkim	...	...	...	15	
Total				4,50,075	4,50,075

Grand Total ... 11,48,349

B. CHOLERA VACCINE.

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

<i>Months.</i>				<i>Quantities in millilitres.</i>	
January	...	...	...	1,28,166	
February	...	...	...	2,47,257	
March	...	...	...	20,660	
April	...	...	...	1,61,208	
May	...	...	...	2,80,403	
June	...	...	...	3,82,323	
July	...	...	...	3,97,517	
August	...	...	...	14,50,747	
September	...	...	...	6,79,567	
October	...	...	...	51,710	
November	...	...	...	12,725	
December	...	...	...	46,891	
Total				38,59,174	

C. T. A. B. VACCINE.

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

<i>Months.</i>					<i>Quantities in millilitres.</i>
January	...	...	...	...	7,789
February	...	...	...	...	12,532
March	...	...	...	...	22,411
April	...	...	...	...	11,016
May	...	...	...	...	5,380
June	...	...	...	...	45,853
July	...	...	...	...	48,932
August	...	...	...	...	41,632
September	...	...	...	...	67,539
October	...	...	...	...	60,376
November	...	...	...	...	44,564
December	...	...	...	...	22,058
Total				...	3,90,082

D. MEDIA SECTION.

The functions of this department are:—

- (1) The production of media for the manufacture of vaccines,
- (2) The production of media for routine work carried out at this Institute,
and
- (3) The production of parenteral solutions.

Owing to less demand for plague vaccine the Department did not prepare any hydrolysate for its manufacture, but for cholera vaccine 13,000 flasks of 500 ml. each of casein hydrolysate were prepared. For the preparation of T. A. B. Vaccine 3,388 Rouxe Bottles (200ml. of agar in acid digest broth in each) were prepared.

Different types of media were prepared both for routine and research work for the various departments of the Institute. For testing the sterility of vaccines, toxins, antitoxins and parenteral fluids, 3,500 bottles each of aerobic and anaerobic media in acid digest broth were prepared.

The parenteral solutions section kept up its steady progress. During the year the prices and packing of all solutions were revised and hence the amount realised was less, although the demand for the solutions was quite high. The figures for the year 1953 were higher due to unprecedented cholera outbreaks in the country.

The following table gives the details of cash sales during the year 1954 :—

Sale of Glucose Saline etc. during 1954.

Serial No.	Name of Product.	Number of bottles sold.	Amount.
			Rs.
1	Glucose Saline 500 cc.	33,539	64,217
2	Hypertonic Saline 500 cc. ..	1,162	2,290
3	Normal Saline 500 cc.	3,787	7,305
4	Protein Hydrolysate 400 cc. ..	8,795	22,622
5	5 per cent. Glucose Solution 500 cc.	15,217	29,030
6	25 per cent. Glucose Solution 100 cc.	2,171	1,363
7	25 per cent. Glucose Solution 500 cc.	2,050	5,125
8	50 per cent. Glucose Solution 100 cc.	829	829
9	Sodium Sulphate 500 cc.	445	840
10	Sodium Citrate 500 cc.	978	1,921
11	Distilled Water 500 cc.	2,689	1,864
12	A. C. D. Solution 500 cc.	512	731
Total ..		72,174	1,38,290

The following table gives comparative figures for previous years :—

Year.	No. of bottles.	Amount in Rs.
1947	10,250	15,986
1948	15,995	20,781
1949	31,718	46,542
1950	47,111	88,838
1951	54,892	1,03,232
1952	67,942	1,28,930
1953	1,00,073	1,94,934
1954	72,174	1,38,137

E. SECTION OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY.

This section mainly carried out the bacteriological and chemical examination of samples of water for ascertaining their fitness for drinking, sanitary and industrial purposes.

These samples were received from the Bombay State as well as from States.

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

<i>Sample examined.</i>	<i>Nature of examination.</i>	<i>No. of samples examined.</i>
Water ..	For drinking purposes ..	82
Water ..	For bathing purposes ..	8
Water ..	Fitness for Sanitary and Industrial purposes.	12
Total ..		102

IV. Department of Antitoxins and Sera.

During the year under review the department made further efforts to increase the production of different antitoxins and sera, since the demands for the same continued to increase from various States and Armed Forces. With the acquisition of the Pimpri Farm, production was stepped up by 50 per cent. in the past two years, utilising the limited available facilities afforded by the Farm. The production which had almost reached a peak last year was this year further increased by about 20 per cent.; the total sera both natural and concentrated produced during the year under review were 1,681,140 ml. as compared to 1,408,100 ml. for the previous year. This was mainly possible due to better immunity response by the horses due to their initial housing under healthier and open air conditions at Pimpri Farm before they were brought down to Bombay for hyperimmunization.

Initial attempts at the production of Antirabies Serum in horses, being encouraging, its production on a regular basis is being organised and it is expected that some quantity could be made available for public use soon. During the year four horses were immunized against Rabies virus and 39,000 ml. of immune plasma was processed and from the last year's stock of mature concentrated sera 287 ampoules of 10 cc. each were lyophilised for tests and trials. Preliminary protection tests on mice with the sera gave a high neutralisation value.

The demands for anti-snake-venom sera are continually increasing and the total of 2,50,220 ml. of lyophilised antivenin was issued during the year as against 1,05,580 ml. of the last year. Even with this $2\frac{1}{2}$ times increase in production all the demands were not fully met. Demands for this sera are received even from foreign countries.

During the year greater demands were received for prophylactic toxoids against diphtheria and tetanus for human immunization. As the consciousness for the use of these important prophylactics, is more apparent, efforts are being made to increase the production of these toxoids for human use. No definite decision was possible regarding the regular manufacture and issue of N. A. F. T. for diphtheria prophylaxis in human beings, since reports about its initial claims were awaited from workers abroad. It was, therefore, decided to prepare purified diphtheria toxoid precipitated by Aluminium phosphate (P. T. A. P.) for distribution.

As the demands were very heavy during the year for various sera, all efforts were mainly directed to produce the maximum quantity possible within the limits of efficiency.

1. *Horses*.—The total number of horses used for producing antitoxins and sera during the year were 183. Besides these there were 50 horses at Pimpri undergoing preliminary immunization at the end of the year. The horses under immunization were grouped as follows :—

<i>Antitoxins.</i>				<i>Number of horses used.</i>
1.	Tetanus Antitoxin	...	...	85
2.	Diphtheria Antitoxin	...	...	44
3.	Anti-snake-venom Serum	...	...	37
4.	Gas gangrene Antitoxin	...	...	9
5.	Anti-Dysentery	...	...	2
6.	Anti-rabic	...	...	4
7.	Normal Horse Sera	...	...	2
Total				183

2. *Preparation of Toxins and Toxoids*.—During the year, diphtheria, tetanus, welchii and Shiga toxins were Produced. The reserve stock of the other toxins sufficed during the year. A part of the diphtheria and tetanus toxins were converted into toxoids for immunization of horses. A portion of the toxoids were also purified for human use.

<i>Types of Toxins and Toxoids.</i>				<i>Quantities in ml.</i>
Tetanus	...	...	...	452,950
Diphtheria	...	...	...	181,900
Welchii	...	...	...	61,500
Septique	...	...	...	Nil.
Oedematiens	...	...	...	Nil.
Shiga	...	...	...	40,000
Tetanus toxin converted into toxoid	...	...	...	49,950
Diphtheria toxin converted into toxoid	...	...	...	12,100

3. *Preparation of Antitoxins and Sera*.—The quantity of each concentrated sera and natural sera are as follows :—

I. CONCENTRATED SERA.

<i>No.</i>	<i>Name.</i>				<i>Amount of concentrated serum in ml.</i>
1	Tetanus Antitoxin	...	...	...	801,100
2	Diphtheria Antitoxin	...	...	...	194,100
3	Antivenin	...	...	...	476,410
4	Gas-gangrene Antitoxin	...	...	...	57,300
5	Anti-Dysentery	...	...	...	8,500
6	Anti-rabic	...	...	...	4,000
Total					1,541,400

II. NATURAL SERA.

No.	Name.	Amount of concentrated serum in millilitres.
1	Normal Horse Serum	65,940
2	Anti-Dysentrey Serum	73,800
Total		1,39,740

4. *Lyophilisation of Sera*.—Polyvalent anti-snake-venom serum was issued in lyophilised form during the year. The amount of serum issued was 2,50,220 cc.

5. *Supply*.—The sale value of the products of this department in 1954 was Rs. 8,73,426 as compared with Rs. 6,77,911 for 1953. The following quantities of antitoxins, toxoids and sera were issued during the year :—

	Quantities supplied in millilitres.
(1) <i>Tetanus Antitoxin</i> —	
(a) Prophylactic 3,000 I. U. in 2 cc.	5,77,046
(b) Curative 10,000 I. U. in 5 cc.	21,915
(c) Curative 20,000 I. U. in 10 cc.	1,11,550
(d) Highly concentrated 20,000 I. U. in 4 cc.	83,756
(2) <i>Diphtheria Antitoxin</i> —	
(a) Prophylactic 1,000 I. U. in 2 cc.	5,382
(b) Curative 5,000 I. U. in 5 cc.	4,485
(c) Curative 10,000 I. U. in 10 cc.	2,36,620
(d) Highly concentrated 10,000 I. U. in 5 cc.	7,945
(3) <i>Gas-gangrene Antitoxin</i> —	
(a) 3,000 I. U. Welchii Antitoxin in 10 cc.	64,270
(b) 9,000 I. U. Welchii 4,500 I. U. Septic and 9,000 I. U. Oedematiens Antitoxins, in a volume not exceeding 15 cc. highly concentrated and purified.	4,815
(4) <i>Anti-plague Serum</i> , 20 cc.	600
(5) <i>Lyophilised Polyvalent Anti-snake-venom Serum</i> , 10 cc.	2,50,220
(6) <i>Anti-dysentery Serum</i> —	
(a) Natural, 20 cc.	460
(b) Concentrated 4,000 I. U. in 5 cc.	305
(7) <i>Tetanus Toxoid</i> :—	
(a) 10 cc.	2,670
(b) 5 cc.	1,090
(c) 1 cc.	1,426

	<i>Quantities supplied in millilitres.</i>
(8) <i>Diphtheria Toxoid</i> :—	
(a) 5 cc. 	3,850
(b) 1 cc. 	1,077
(9) <i>Normal Horse Serum in 10 cc.</i>	5,070

Total 13,74,439 ml. of antitoxins and sera and 10,113 ml. of toxoids.

In addition to the above, 34,140 ml. of natural horse serum was supplied to local hospitals.

Training.—During the year Shri Mohommad Omar Khan, W. H. O. Fellow from Afghanistan received training in the production of Antitoxins and Sera.

V. Department of Entomology.

Technical assistance was given to the different States of the Indian Union, Local Bodies and private individuals in respect of their problems on Fleas, Rats, Rat-traps and insects of medical importance. Several enquiries from different Institutions and private bodies were attended to in connection with insect pests, scorpions, centipeds, spiders and earthworms.

A course in Plague preventive measures was held from 15th February 1954 to 2nd March 1954. Twenty-four epidemic medical officers and sanitary inspectors deputed by the Director of Public Health, Bombay, Saurashtra and a number of municipalities attended the course.

Nearly 3,000 persons belonging to the various academic institutions and private bodies attended the talks given on snakes and witnessed the snake venom extraction.

This department assisted in setting up exhibits for the Institute at four public exhibitions held at various places in Bombay.

The work on the collection of rat-ectoparasites was concluded. These studies have shown that *X. cheopis* is predominant on *Rattus rattus* and *X. astia* on *B. bengalensis*. These observations are also being carried out in the field.

The Entomological Survey studies of the Swadeshi Mill area, Kurla, were concluded during the year. A number of nematodes have been recovered from *Culex fatigans*. The breeding of *Culicoides* was observed not only in the mill area, but also in similar habitats in the island of Bombay. Control measures for *Culicoides* in particular, and mosquitoes in general, were tried and recommendations made for the use of insecticide impregnated bricks in stagnant pools, and the spraying of a mixture of DDT and BHC water dispersible suspension in the houses.

In the studies on the "Pulicidal value of Pyrethrum", the deterioration of this Insecticide in light and dark was studied. Boosters like piperonyl butoxide, sesame oil, castor oil and stabilisers like Hydroquinone and Pyrogallol have been used to see if there is any check in this reduction of the Pyrethrins. Potter's tower was designed to dust the various powder formulations on a standard basis, and these standard formulations tested for the first time in open rat burrows in godowns.

Experiments were done on behalf of the W. H. O. to find out if the body-lice prevailing in and near Bombay showed any resistance to DDT. Two trials lasting for a period of 10 days each have so far been made on the lice obtained from nearby hospitals and receiving centres for destitutes around Bombay.

Biometrical studies on the different common rats of Bombay were started with the object of getting further information on the characteristics of these rats.

Rodenticidal properties of Ratex, a proprietary Red-squill derivative were evaluated on a comparative basis with Barium carbonate.

Four post-graduate students in Entomology and two in herpetology were guided for their degrees of the University of Bombay.

A. SNAKES.

Seventy-two specimens of snakes and lizards were received for identification. The specimen were brought by institutions and private bodies from the island of Bombay and from other parts in the State.

In the collection of snake-venom, more attention was devoted to procure Krait and *Echis* venom. The following is the statement showing the production and supply of different venoms expressed in gms. during the year.

Cobra.		Russell's viper.		Krait.		Saw-scaled viper.	
Collected.	Supplied.	Collected.	Supplied.	Collected.	Supplied.	Collected.	Supplied.
60.7 gm	62.8 gm	64.1 gm	63.0 gm	20.1 gm	4.9 gm	23.2 gm	13.4 gm

The snake farm whose operation was started last year worked satisfactorily. Cobra, Krait and Russell's viper snakes were housed in the farm in hide-outs most suited to their habits. The average venom output from snakes in the farm has been found to be higher than the ones kept in cages in the laboratory.

Experiments are being conducted to study the sexual cycle in these snakes with particular reference to the hibernation of spermatozoa if any, in the oviducts.

Field observations were conducted at Deogad (a place from where we get all the Saw-scaled vipers) to study the food habits of *Echis Carinatus*, as it has been found difficult to keep these snakes in captivity for long in the Institute. The collection of the Fauna under stones and its comparison with the gut contents of *Echis* dissected on the spot shows that the majority of these are insects. Further field observation in winter and summer are to be made to see whether there is any aestivation in this snake and whether there is any change in the food.

B. EXAMINATION OF RATS FROM BOMBAY CITY AND PORT TRUST AREA.

A permanent organisation is maintained by the Municipality of Bombay for catching rats. On an average 2,575 rats were received every day at the Institute from the different wards within the Municipal area. These rats were either trapped alive or found dead in the various parts of the City including the Port Trust area. All the rats were classified according to species. Only the dead ones were dissected and examined for post-mortem signs of plague infection. In doubtful cases, diagnosis was confirmed by smear and cultural examination. All the rats received daily were finally destroyed by incineration. Besides providing valuable information on the subject of epidemiology, this work is intended to give the Municipal authorities, the first signal of the plague epizootic in any part of the City with a view to controlling plague infection in time. During the year under review, no plague cases were reported in Greater Bombay.

Experiments on the susceptibility of Bombay City rats to plague infection by injecting a standard test infective dose were continued during this year also. The following results were obtained :—

Species of rats.	No. of rats used in experiments.	No. of rats dead.	Mortality per cent.
<i>R. rattus</i> ...	89	11	12.1
<i>R. norvegicus</i> ...	89	3	3.3
<i>Gunomys kok</i> ...	56	43	76.7

The results do not show any significant difference as compared to the results of of last year.

C. EXAMINATION OF RATS FROM STEAMERS AND OTHER SOURCES.

During the year under review, plague epidemic was apprehended in some of the districts of State due to suspected rat-fall. As a result of this, a large number of smears and flea samples were received for examination. In all 16 dead rats, 267 smears from dead rats and 13,390 fleas were received from different towns of the Bombay State. Two dead rats and 4 smears were also received from other States. No plague infection was detected. In the same year, 355 killed rats were received from the Health Officer of the Port of Bombay. Of these, 313 belonged to the species of *Rattus rattus rufescens* 9 *Rattus norvegicus*, 4, *Gunomys kok* and 29 *Mus dubius*. None of them was found plague-infected.

During the year under review, 15 rats were received from the City of Bombay through other agencies or private persons. None of them was found plague-infected.

TABLE I.—*Showing rats received at the Institute from the Bombay Municipality during 1954.*

Month		Alive	Dead.	Total.
January		88,912	45	88,957
February		77,421	57	77,478
March		78,724	51	78,775
April		75,933	51	75,984
May		72,391	37	72,428
June		76,786	50	76,836
July		76,926	75	77,001
August		76,618	35	76,653
September		68,137	60	68,197
October		74,920	45	74,965
November		82,147	34	82,181
December		89,903	26	89,929
Total		9,38,818	566	9,39,384

TABLE II.—*Showing dead rats examined for plague during 1954.*

Month		Dead rats examined.	Found plague infected.
January		27	Nil.
February		30	"
March		26	"
April		30	"
May		14	"
June		31	"
July		20	"
August		24	"
September		56	"
October		45	"
November		30	"
December		24	"
Total		357	Nil.

In addition to dead rats, 10½ live rats from plague suspected localities and 6,587 live rats from the Bombay Port Trust area were examined during the year. The total number of live and dead rats examined is 7,048.

TABLE III.—Showing the number and percentage of different species of rats trapped alive by Bombay Municipal Corporation during 1954.

Species.	January		February.		March.		April.		May.		June.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus rufescens</i> ...	22,879	25·8	19,618	25·3	17,745	22·5	15,927	21·0	15,159	20·9	15,077	19·6
<i>Rattus norvegicus</i> ...	16,369	18·4	13,361	17·3	13,210	16·8	12,016	15·8	12,817	17·7	13,278	17·8
<i>Gunomys kok</i> ...	30,939	34·8	29,461	38·1	30,060	38·2	32,008	42·1	29,683	41·0	33,389	43·5
<i>Bandicota malabarica</i> ...	1,021	1·1	744	0·9	559	0·7	506	0·7	563	0·8	813	1·1
<i>Mus dubius</i> ...	6,448	7·3	4,606	5·9	5,770	7·3	4,379	5·8	3,394	4·7	3,374	4·4
<i>Suncus caeruleus</i> ...	11,256	12·6	9,631	12·5	11,380	14·5	11,097	14·6	10,775	14·9	10,855	14·1
Total ...	88,912	100·0	77,421	100·0	78,724	100·0	75,933	100·0	72,391	100·0	76,786	100·0

Species.	July.		August.		September		October		November.		December.		Whole year.	
	Number.	Percentage.	Number	Percentage.	Number	Percentage.	Number	Percentage.	Number	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus rufescens</i> ...	19,253	25·0	18,862	24·6	17,203	25·3	21,533	28·7	23,119	28·2	25,481	28·4	2,31,861	24·7
<i>Rattus norvegicus</i> ...	14,175	18·4	14,332	18·7	13,241	19·4	13,473	18·0	14,392	17·5	16,786	18·7	1,67,450	17·8
<i>Gunomys kok</i> ...	28,533	37·1	29,576	38·6	25,214	37·0	26,121	34·9	29,510	35·9	31,939	35·5	3,56,433	38·0
<i>Bandicota malabarica</i> ...	726	1·0	673	0·9	541	0·8	577	0·8	625	0·8	791	0·9	8,139	0·9
<i>Mus dubius</i> ...	3,375	4·4	3,322	4·3	3,129	4·6	4,310	5·7	5,029	6·1	5,432	6·0	52,568	5·6
<i>Suncus caeruleus</i> ...	10,864	14·1	9,853	12·9	8,804	12·9	8,906	11·9	9,472	11·5	9,474	10·5	1,22,367	13·0
Total ...	76,926	100·0	76,618	100·0	68,137	100·0	74,920	100·0	82,147	100·0	89,903	100·0	9,38,818	100·0

TABLE IV.—Showing the number and percentage of different species of rats found dead in the City of Bombay (including the suburbs) during 1954.

Species.	January.		February.		March.		April.		May.		June.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus rufescens</i> ...	12	26·7	14	24·5	7	13·7	13	25·5	5	13·5	18	36·0
<i>Rattus norvegicus</i> ...	11	24·4	7	12·2	11	21·6	9	17·6	8	21·6	...	...
<i>Gunomys kok</i> ...	4	8·9	8	14·3	8	15·7	8	15·7	1	2·7	13	26·0
<i>Bandicota malabarica</i> ...			1	1·7	...	...	...	...	...	...	...	...
<i>Mus dubius</i> ...					...	...	...	...	...	...	...	...
<i>Suncus caeruleus</i> ...			2	3·5	...	...	...	...	...	...	2	4·0
<i>Putrid</i> ...	18	40·0	25	43·8	25	49·0	21	41·2	23	62·2	17	34·0
Total ...	45	100·0	87	100·0	51	100·0	51	100·0	37	100·0	50	100·0

Species.	July.		August.		September.		October.		November.		December.		Whole year.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus rufescens</i> ...	13	17·3	8	22·9	27	45·0	21	46·7	12	35·3	6	23·1	156	27·6
<i>Rattus norvegicus</i> ...	5	6·7	4	11·4	17	28·3	13	28·9	7	20·6	6	23·1	98	17·3
<i>Gunomys kok</i> ...	2	2·7	12	34·3	10	16·7	8	17·8	11	32·3	10	38·4	95	16·8
<i>Bandicota malabarica</i> ...	...	...	...	...	...	...	1	2·2	...	...	...	...	2	0·3
<i>Mus dubius</i> ...	...	...	...	...	2	3·3	2	4·4	...	...	2	7·7	6	1·1
<i>Suncus caeruleus</i> ...	...	...	...	...	...	...	...	...	...	...	...	...	4	0·7
<i>Putrid</i> ...	55	73·3	11	31·4	4	6·7	...	...	4	11·8	2	7·7	205	36·2
Total ...	75	100·0	35	100·0	60	100·0	45	100·0	34	100·0	26	100·0	566	100·0

EXAMINATION OF RATS FROM THE BOMBAY PORT TRUST DOCKS.

These rats were received along with those of the other wards of the Bombay Municipality, and have been already accounted for in the monthly totals given in previous tables. A separate record of these rats, giving the number and percentage of the different species, is also maintained at the instance of the Public Health Commissioner.

TABLE V.—Showing the number and percentage of different species of rats caught alive from the Bombay Port Trust area in 1954.

Species.	January.		February.		March.		April.		May.		June.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus rufescens</i> ...	559	68·3	266	50·8	280	62·2	299	65·0	202	40·5	318	53·4
<i>Rattus norvegicus</i> ...	66	8·0	88	16·8	68	15·1	32	7·0	84	16·8	75	12·6
<i>Gunomys kok</i> ...	69	8·4	58	11·1	40	8·9	72	15·7	106	21·3	128	21·5
<i>Bandicota malabarica</i> ...	12	1·5	5	0·9	5	1·2	3	0·6	8	1·6	6	1·0
<i>Mus dubius</i> ...	51	6·2	23	4·4	24	5·3	28	6·1	60	12·0	33	5·5
<i>Suncus caeruleus</i> ...	62	7·6	84	16·0	33	7·3	26	5·6	39	7·8	36	6·0
Total ...	819	100·0	524	100·0	450	100·0	460	100·0	499	100·0	596	100·0

Species.	July.		August.		September.		October.		November.		December.		Whole year.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus rufescens</i> ...	308	55.4	327	58.3	348	51.2	323	59.4	362	61.6	402	51.4	3,994	56.5
<i>Rattus norvegicus</i> ...	97	17.4	94	16.8	81	11.9	42	7.7	74	12.6	119	15.2	920	13.0
<i>Gunomys kok</i> ...	86	15.5	45	8.0	137	20.2	52	9.6	39	6.6	120	15.3	952	13.5
<i>Bandicota malabarica</i> ...	...	...	3	0.5	15	2.2	4	0.7	5	0.8	14	1.8	80	1.3
<i>Mus dubius</i> ...	22	4.0	51	9.1	73	10.7	96	17.6	81	13.8	99	12.7	641	9.0
<i>Suncus caeruleus</i> ...	43	7.7	41	7.3	26	3.8	27	5.0	27	4.6	28	3.6	472	6.7
Total ...	556	100.0	561	100.0	680	100.0	544	100.0	588	100.0	782	100.0	7,059	100.0

TABLE VI.—*Showing the different localities in the Bombay Province from which smears, rats and fleas were received for examination during 1954.*

Name of Town.	No. of smears.	No. of rats.	Fleas.	
			No. of samples.	No. of fleas.
Ahmednagar ..	2	..	..	..
Baramati ..	..	..	18	685
Bijapur ..	6	2	11	1,646
Bassein ..	..	1	..	..
Dangs, Ahuwa ..	6	..	..	..
Hubli ..	1	..	..	..
Karad ..	2	..	..	..
Colaba ..	..	1	..	..
Malad ..	..	1	..	..
Panvel ..	..	2	..	..
Panch Mahals, Godhra	8	6	..	..
Palghar ..	..	1	..	..
Sindagi ..	1	..	..	..
Satara ..	21	1	92	8,196
Sholapur ..	220	..	15	2,863
Thana ..	..	1	..	..
Total ..	267	16	136	13,390

No plague infection was detected from the examination of smears, rats and fleas mentioned above.

TABLE VII.—*Bombay City rats supplied to different Institutions during the year 1954.*

Name of the Institution.		No. of rats supplied.	
1.	Wilson College ..	..	312
2.	Ramnarain Ruia College ..	..	260
3.	Institute of Science ..	..	310
4.	Jai Hind College ..	..	103
5.	Ruparel College ..	..	41
6.	Elphinstone College ..	..	4
7.	Siddharth College ..	..	18
8.	Seth G. S. Medical College ..	..	14
9.	Khalsa College ..	..	12
10.	Prince of Wales Museum of Western India ..	..	2
Total ..			1,076

VI. Department of Clinical Pathology and Diagnostic Reagents.

The Department of Clinical Pathology and Diagnostic Reagents continued to (1) conduct microscopic, bacteriological, serological and biological tests on clinical materials obtained from Government hospitals, and dispensaries as well as from private practitioners and institutions; (2) prepare, stock and issue diagnostic reagents and stains; (3) carry out histological examination of research and biopsy material and pathological tissues; (4) prepare autogenous vaccines; (5) conduct special tests such as Rideal Walker Tests and the tests for pregnancy, and (6) conduct research.

A. GENERAL BACTERIOLOGICAL WORK.

Clinical material from the various Government hospitals and dispensaries from the City of Bombay and outside stations as well as from private practitioners and institutions continued to be received for examination during the year under review. All essential tests for the diagnosis of infectious diseases such as diphtheria, cerebrospinal fever, enteric group of fevers, typhus, cholera, etc., were carried out free of charge.

(i) *The Government of Bombay Typhoid Scheme.*—The Scheme was continued for the seventh year and found very popular and useful. 752 calls from private practitioners were attended to. As before, blood samples were collected for blood culture and triple Widal tests. Some practitioners required additional laboratory tests which were carried out for their convenience on payment. All cases positive for typhoid group were reported to the Executive Health Officer, Bombay Municipality, Bombay, to enable him to take the necessary preventive measures at the earliest opportunity. In this examination of 752 blood samples for culture and Widal, 227 i.e. 30 per cent. were positive. An analysis of records from the Section showed the following interesting points:—

(a) *Seasonal Incidence of Typhoid in Bombay.*—The largest number of calls were during the month of June to October with lower incidence in the month of January to March.

(b) *Results of blood culture and triple Widal Test.*—Samples examined during 1952, 1953 and 1954 are tabulated below to show the type and extent of typhoid infection amongst cases of fevers of unknown origin:—

Year.	Total samples.	Hospital samples.	Typhoid Scheme samples.	Widal— Culture+	Widal+ Culture—	Widal+ Culture+	Total B.typho- Cultures done.	B.para A.sus iso- lated.	B. para A. isolated.
1952	... 4,189	3,355	834	161	646	151	4,189	366	57
1953	... 3,416	2,651	765	113	680	62	3,385	161	46
1954	... 3,503	2,776	727	111	597	69	3,476	196	52

(ii) *Venereal Disease Reference Laboratory*.—This department continued to work as a Reference Laboratory on behalf of W. H. O. for venereal diseases control programme. Small quantities of V. D. R. L. and Meinicke's antigens were received from W. H. O. and a few representative samples of tested sera from some venereal clinics from the City of Bombay were obtained and checked. These clinics discontinued sending samples from 1st August 1954. In all 448 samples were tested. Of these, there was agreement with our results to the extent of 97·5 per cent. Arrangements are underway to include more, and, finally all venereal clinics in the State of Bombay. Most of the venereal clinics in the State are using Standard Kahn Antigen prepared by this Institute.

(iii) *Routine Examination*.—The number of routine examinations carried out during the years 1952, 1953 and 1954 are shown in the following table for purposes of comparison:—

		1952.	1953.	1954.
I. <i>Serological Examinations</i> —				
(1) Wassermann Test	..	3,661	3,004	3,664
(2) Kahn Test	..	2,627	2,935	2,620
(3) Triple Widal Test	..	2,687	2,568	2,415
(4) <i>Agglutination Test for</i> :—				
(a) Typhus	..	3,142	2,568	2,415
(b) Brucellosis	..	2,811	2,573	2,422
(c) Leptospirosis	..	64	144	58
(d) Paul-Bunnell Test	..	19	9	9
II. <i>Bacteriological and Biological Tests</i> —				
(1) Blood culture for enteric group.		4,189	3,385	3,476
(2) Urine culture for pathogenic organisms.		67	123	78
(3) Throat swabs for diphtheria.		238	281	405
(4) Stools and rectal swabs for culture.		1,152	1,680	65
(5) Sputum and body fluids for AFB.		733	961	870
(6) C. S. F. for culture	..	461	425	504
(7) Material from suspected plague cases.		14	Nil	Nil
(8) Material from Medico-legal Cases.		33	25	13

	1952.	1953.	1954.
III. <i>General Microscopic Examinations</i> —			
(1) Blood films for M. P. and Filariasis.	54	25	27
(2) Blood cell count ..	1,621	1,942	1,763
(3) Bone Marrow	6	26	8
(4) Semen for fertility ..	56	43	83
(5) Material for smear examination (Presence of pathogenic organisms like G. C., etc.).	51	55	46
(6) Stool—Routine test ..	1,068	1,019	1,062
(7) Urine—Routine test ..	890	1,114	1,203
IV. Pregnancy Test	523	691	841
V. Rideal Walker Test ..	47	68	53

(iv) *Autogenous Vaccines*.—Use of autogenous vaccines is still in vogue but to a lesser extent amongst the practitioners in Bombay and outstations. Cases of chronic inflammatory conditions and allergic states such as sinusitis, follicular pharyngitis, gingivitis, bronchial asthma, etc. etc. applied for such vaccines. The number of courses issued during 1952, 1953 and 1954 are shown below :—

	1952.	1953.	1954.
Courses of autogenous vaccines supplied.	55	28	35

B. DIAGNOSTIC REAGENTS.

(i) *Supply of Diagnostic Reagents*.—The work in connection with the production, stocking and supply of various reagents for diagnostic purposes to laboratories all over the State of Bombay and outside the State was continued.

<i>Reagents—</i>	<i>Quantity supplied in ml.</i>		
	1952.	1953.	1954.
(1) Killed suspensions for Widal and Weil-Felix Tests.	1,60,485	1,64,895	1,60,215
(2) Blood Grouping Sera ..	7,170	7,776	8,751
(3) High Titre Sera and Amboceptor.	756	686	676
(4) Kahn Antigen	5,825	4,932	4,980
(5) W. R. Antigen	350	180	330
(6) Russell's viper and Cobra Venom solutions.	2,893	1,436	2,545

(ii) *Supply of Stains*.—In addition to the above diagnostic reagents, the supply of various biological stains was also continued. There is an increasing demand for these stains, as is evident by the supply of 1,61,555 ml. this year as against a total of 1,13,960 ml. last year.

(iii) *Collection of Standard Strains*.—Collection of standard strains of different Pathogenic organisms is continued. A number of demands for such strains, for demonstration purposes from various teaching institutions were met during the year.

C. HISTOPATHOLOGY SECTION.

Pathological Specimens.—During the year under review, 2,133 pathological specimens received from different hospitals in the State of Bombay and a few from outside the State were examined and reported upon. These included neoplasms, gynaecological conditions, tuberculosis of different organs and some rare and interesting pathological specimens. In addition, 478 research specimens from experimental animals were processed. These were mostly from different departments of the Institute as well as different research enquiries conducted at the Institute. In all, 2,611 specimens were subjected to histological examination.

The classification of the specimens of tissues received from hospitals in the City of Bombay and outstations during the years 1952, 1953 and 1954 is tabulated below :—

	1952.	1953.	1954.
<i>Classification—</i>			
(1) Number of samples found to be normal.	284	265	185
(2) Number of samples unfit for examination.	136	157	140
(3) Samples for miscellaneous pathological conditions.	884	1,172	1,366
(4) Samples for tuberculosis infection.	123	138	95
(5) Non-malignant Neoplasms ..	65	95	125
(6) Malignant Tumours ..	114	146	158
(7) Brain samples for Negri Bodies	79	83	64
Total ..	1,685	2,056	2,133

It will be seen that there has been a steady increase in the number of samples received in the department for histological examinations.

Some of the important and interesting pathological conditions encountered are grouped and tabulated below :—

I. NEOPLASMS.

(a) *Malignant* :—

(1) Carcinoma Primary—

Adenocarcinoma	..	..	..	18
Anaplastic carcinoma	..	..	..	1
Arrhenoblastoma	..	..	..	1
Basal cell carcinoma	..	..	..	2
Carcinoid ..	..	..	..	1
Duct carcinoma	..	..	..	1
Epidermoid carcinoma	..	..	..	26
Medullary carcinoma	..	..	..	4
Mucoid carcinoma	..	..	..	3
Papillary carcinoma	..	..	..	1
Scirrhus carcinoma	..	..	..	2
Squamous cell carcinoma..	..	..	..	10
Transitional cell carcinoma	..	..	..	1
Carcinoma simplex	..	..	..	35

(2) Carcinoma Secondary 9

(3) Sarcoma—

Chondrosarcoma	..	..	..	1
Fibrosarcoma ..	..	..	..	4
Giant cell sarcoma	..	..	..	1
Melanotic sarcoma	..	..	..	1
Neurogenic sarcoma	..	..	..	1
Reticulum cell sarcoma	..	..	..	1
Round cell sarcoma	..	..	..	1
Sarcoma ..	..	..	..	9

(4) Malignant Lymphoma—

Hodgkin's sarcoma	..	..	..	3
Lymphosarcoma	..	..	..	4

(5) Teratoma 4

(6) Seminoma 1

(b) *Non-malignant Neoplasms* :—

Adenoma	..	..	..	..	5
Adenomyoma	..	..	..	..	1
Angioma	..	..	..	..	2
Cyst-adenoma	..	..	..	..	3
Colloid Goitre	..	..	..	..	1
Diffuse Goitre	..	..	..	..	2
Fibro-adenoma	..	..	..	..	10
Fibrolipoma	..	..	..	..	6
Fibroma	..	..	..	:	47
Fibroma cellular	..	..	..	..	2
Fibrous Epulis	..	..	..	..	2
Haemangioma	..	..	..	..	3
Haemangioma capillary	..	..	..	..	5
Lipoma	..	..	..	..	7
Mixed Parotid Tumour	..	..	..	..	7
Neurofibroma	..	..	..	..	2
Osteoclastoma	..	..	..	..	1
Osteochondroma	..	..	..	..	1
Papilloma	..	..	..	..	9
Papilliferous cyst-adenoma	..	..	..	..	1
Pseudomucinous cyst-adenoma	..	..	..	..	2
Rhinoscleroma	..	..	..	..	1
Rhinosporidiosis	..	..	..	..	1
Scleroderma	..	..	..	..	1
Serous cyst-adenoma	..	..	..	..	3

II. TUBERCULOSIS IN DIFFERENT ORGANS.

Breast	..	..	..	..	2
Female genital tract	..	..	..	..	49
Lymphnodes	..	..	..	..	22
Intestinal tract	..	..	..	..	9
Other tissues	..	..	..	..	13

An interesting set of specimens received during the year was specimen Nos. 1973 to 1984 of 9th December 1954. Sections of viscera from a male aged 19 years showed evidence of testis, vas, ovary, fallopian tubes, uterus. Sections of intra-abdominal tumour showed appearances of Embryonal Carcinoma. The samples were received after partial-post-mortem examination. Further clinical details are awaited.

D. SPECIAL INVESTIGATIONS.

(i) *Epidemic Jaundice in Phoenix Mills, Bombay.*—During the month of August 1954, some cases of Jaundice were reported from Phoenix Mills, Bombay.

Routine cultural and biochemical examinations of blood, urine and stool were carried out in a representative group of 7 cases. Agglutination, cultural and guinea-pig inoculation tests were also carried out to detect evidence of leptospiral infection. None of the samples showed positive reaction for leptospirosis. The collective data of various tests showed evidence of Infective hepatitis (Catarrhal jaundice) as the aetiological basis. No mortality was reported.

(ii) *Inter Laboratory Evaluation of Serologic Tests for Syphilis.*—This department continued to co-operate in this Enquiry of the Indian Council of Medical Research. 575 samples of sera were obtained in 40 batches. As before, each batch of sera was subjected to quantitative and qualitative Wassermann, Kahn and V. D. R. L. tests, thus involving a great deal of work.

50 dried sera sent by W. H. O. were also examined by quantitative and qualitative V. D. R. L. slide test.

(iii) *Hook-worm Survey in Kaira District.*—A laboratory unit was sent to make a survey of Hook-worm infestation in Kaira District. This investigation was taken up in response to report sent by local medical practitioners who suspected about 34 per cent. incidence of Hook-worm disease in various villages of the Kaira District.

The survey work is in progress.

E. TRAINING AND RESEARCH SCHOLARS.

The following research scholar worked in the department :—

Shri S. M. Vaidya continued during the year on the subject of Antibiotic Properties of some Indigenous Plants.

The following trainees devoted their time to learn the latest methods used in clinical pathology and acquired knowledge from practical work done in the department :—

(1) Dr. Omar Khan—W. R. O. Fellowship—31st December 1953 to 20th June 1954.

(2) Kumari Kamala R. Gurbuxani, B.Sc.—1st May 1954 to 31st October 1954.

VII. Department of Pharmacology.

During the year under review the pressure of routine work was heaviest of any of the preceding years. Tightening up of the drug control measures and diversion of a large number of samples to this laboratory by the Prohibition Department contributed to this increase. Nevertheless, despite the difficulties, several problems of interest were investigated.

Research Work.—For the last sixty years attempts have been made from time to time to find out a method by which a disease resembling human cholera could be produced in a laboratory animal. In fact, fundamental researches and chemotherapeutic investigations upon cholera have been seriously handicapped by the absence of a suitable laboratory technique. A method has now been developed for inducing cholera in infant rabbits. The disease resembles the human infection in the closest possible way. Chemotherapy of cholera has come within the domain of laboratory investigation since a method for evaluation of cholericidal drugs has also been standardised.

Methods available for the determination of saccharin present in prepared tea (decoction with milk) have been found to be unreliable. A satisfactory method for the detection of saccharin in prepared tea has been worked out. The method involves the extraction of saccharin from tea, its purification and conversion to phenolsulphonephthalein. The latter in alkaline solution yields a red-violet colour which is measured spectrophotometrically.

Reserpine, the hypotensive and sedative principle present in *Rauwolfia serpentina Benth* has been isolated in good quantities from the 'oleoresin' fraction of the alcoholic extract of the root. A simple and economical method has been worked out for the manufacture of Reserpine.

Protein hydrolysate is used intravenously in cases where the patients are unable to assimilate protein orally. The antigenic substances which this hydrolysate may contain have raised the problem of anaphylaxis in patients in whom repeated and intermittent administration of the drug is necessary. A test has been devised on guinea-pigs for determining the antigenicity of the acid digest of casein used for parenteral administration.

Summarised statements of the research work carried out are given in the part II of this report.

Analytical Work.—During the year, 2,361 samples which consisted of pharmacopoeial preparations, non-pharmacopoeial products, patent and proprietary medicines, indigenous drugs, chemicals, surgical dressings, glass wares, etc. were examined. As compared to the last year there was about 20 per cent. increase in the number of samples tested. The following tables give the comparative figures of the total number of samples examined during the last three years :—

	1952	1953	1954
No. of samples tested ..	1,524	1,909	2,361

The drugs were received from various sources and under different categories. These are shown in the table given below :—

Samples received under the Drugs Act.						Samples received from		
Bombay.	Delhi.	U. P.	M. P.	E. P.	Bihar.	Government *Departments.	Commercial firms.	Total.
1066	156	95	20	111	5	644	264	2,361

* Includes samples from the Haffkine Institute.

For the first time, a large number of samples of Tinctures was sent by the Prohibition Department for the purpose of test and analysis and for opinion whether they conform to the prescribed standards. Certain Pharmaceutical firms have been taking advantage of the lack of adequate pharmacopoeial standards by marketing coloured alcohol in the name of spirituous medicinal preparation. This is a serious hindrance for the proper enforcement of prohibition in this State.

Out of the total of 2,361 samples analysed, 36 per cent. were found to be of non-standard quality which showed an increase to the extent of about 9 per cent. over the figure of the previous year. The rise in this figure was caused chiefly by high percentage of sub-standard tinctures received from the Prohibition Department and also similar samples tested under the Drugs Act.

Under the Drugs Act, 1453 samples were examined, out of which 633 were found to be of non-standard quality, the percentage being 44. This compares very unfavourably with the figures of 1953 and 1952, which were 35 and 37 per cent. respectively. As mentioned before, the high percentage of the substandard spirituous medicinal preparations helped in inflating the overall percentage.

During the year under review the number of drugs tested under Schedule C and C (1) of the Drugs Act amounted to 1,123 that was 47 per cent. of the total number of samples. About 24 per cent. of the drugs were found to be of non-standard quality.

A brief review of the reasons for failure of various drugs to comply with the required standards is given below :—

Examination of drugs by chemical methods.

Stability of ergot alkaloids in liquid preparation still remains a problem with the manufacturer. Out of the 11 samples of Ext. Liquid examined, 9 were declared to be of non-standard quality as the contents of alkaloid in them were found to be much lower than the prescribed limits. Only in one sample, the total alkaloid content was observed to be higher.

Injection of Calcium Gluconate in few instances failed to comply with the requirements for the content of calcium and also for clarity. Occasionally, crystalline deposits were noticed.

Samples of Normal Saline in several cases had to be rejected as they did not comply with the official requirements for the content of Sodium Chloride, clarity or absence of Pyrogens. Parenteral solutions of Dextrose or Dextrose Salines in general have been found to be of good quality.

Eight samples of diamino-diphenyl sulphone were analysed during the year, out of which in 4 the active ingredient was less than the stated amount.

Injection of Sodium Morrhuate failed to comply with pharmacopoeial requirements for the limit of alkalinity, and for the content of the active ingredient. It appears that an excess of alkali has been used to solubilise morrhucic acid. This excess alkali interferes with the assay results giving an apparently high figure.

Over 700 spirituous preparations were analysed. These comprised mainly of official Tinctures like Podophylli, Calumbae, Calendulae, Tolutana, Alstoniae, Euonymi, Myrrh, Saussureae, Carminative, Zingiberis, Benzoin, Cubebae, Buchu etc. and extracts of Kolae, Glycerrhiza, etc. Analysis of these tinctures presented certain difficulties. For majority of these preparations, the standard books of reference lay down only the limits of alcohol contents and "weight per ml." in some cases. It is evident that these inadequate official standards are intended mainly for quality control taking it for granted that the tinctures are prepared as per procedure laid down in the official monographs. It was soon realised that majority of the samples of tinctures would have to be declared as of standard quality if one applies the official tests only though obviously they were of non-standard quality. They appeared to be alcoholic preparations containing lower percentage of the active ingredients or simple alcohol to which a little colour or flavouring essence has been added. Therefore, wherever possible, special tests were applied to demonstrate whether the sample was prepared from genuine raw material. Tinctures of Calumbae, Podophylli, Myrrh, Euonymi and Chiretæ are noted for their bitter taste. Similar samples tested here showed hardly any bitterness. For example, out of the 31 samples of Tinct. Calumbea B.P. '48 tested, 28 did not taste bitter. In the case of Tinct. Cubebae which is an alcoholic extract of tailed pepper, the taste of the sample is very characteristic. Out of the 64 samples which were claimed to be Tr. Cubebae, 57 did not taste pepper-like. Majority of the samples of Tinct. Alstonia B.P. 1914 was found to be of non-standard quality, as they did not show any evidence for the presence of alkaloid. About 50 per cent. of the samples of Tr. Buchu failed to comply with the official requirement for weight per ml. at 20° and in some cases for the content of total solids. Sixty-six per cent. of the samples of Tinct. Carminative and 33 per cent. of Tinct. Krameriae also were declared as not of standard quality as they did not comply with the requirement of weight per ml. and in some cases for the content of alcohol.

Out of a dozen samples of absorbent cotton wool examined, 8 failed to comply with the pharmacopoeial requirement for absorbency. Amongst other surgical dressings tested, some samples of absorbent lint failed to comply with the requirements for absorbency and superficial area per ounce. Samples of surgical dressing labelled as "sterilised" were found on examination to be non-sterile.

Examination of drugs by biochemical and microbiological methods.—In this category were included vitamins, insulin and enzymatic preparations. Most of the vitamin preparations were complex in nature in the sense that they contained multiple active ingredients. In addition to different vitamins, they contained amino acids, extracts of yeast, liver, stomach, duodenum, and red bone marrow, mineral salts and in a few cases alkaloids. In some samples 18-20 ingredients were incorporated. Generally speaking, chemical methods were followed for the assay of vitamins. Whenever chemical methods were not applicable, microbiological techniques were applied. For instance, Calcium and Sodium pantothenates, Nicotinic Acid and its amide, vitamin B₁₂ were estimated microbiologically, occasionally microbiological technique was applied for Riboflavin.

Out of the 93 samples tested for their vitamin A content 30 per cent. were found to be of non-standard quality. These were mostly aqueous multivitamin preparations.

Another vitamin which so often failed to comply with the stated amounts was Pantothenate of Sodium and Calcium. Out of 101 preparations containing this vitamin, 38 samples did not comply with the stated amount. In most of these cases vitamin contents were very low and in a few preparation it was totally absent.

It seems, manufacturers are now adding excess quantities of vitamin B₁ than the labelled amount in oral multivitamin preparations. This has rightly been done to counter-act the loss of potency of aneurine hydrochloride due to aging. It has resulted in the improvement of the quality of the preparations during the year under review. Even then, out of the 278 oral preparations containing vitamin B₁ about 16 per cent. failed to comply with the stated amount.

Stability of vitamin C specially in oral multivitamin mixtures, has been a problem for the manufacturer and the analytical data bear testimony to it. Our previous work has demonstrated the instability of the substance under certain conditions.

Sixty-two samples of vitamin B₁₂ were analysed. In 12 the requisite amount of the vitamin could not be obtained. The vitamin has been shown to be adversely affected on exposure to air and in the presence of other substances. It is necessary that manufacturers should carry out investigation on the stability of the substance when mixed with other ingredients.

Examination of drugs by pharmacological methods.—Liver extracts for parenteral administration may contain varying amounts of histamine or histamine like substances. Several samples were referred to this laboratory for determining the amount of histamine present in them. It was observed that the histamine contents of different liver extracts varied widely from a few to 135 μ g per ml. depending on the process of manufacture.

The rat uterus technique remained the method of choice for evaluation of the potency of Ext. Pituitary (Post-lobe) Liquid. The results were frequently checked by the classical guinea-pig uterus method. Out of the 6 samples of pituitary extracts tested only 1 failed to comply with the stated amount.

Forty samples of Adrenaline were assayed biologically, out of which only 3 showed lower potency than the declared amounts. In some samples, Adrenaline was mixed with Procaine or Ephedrine or Pituitary. In these cases chemical and biological tests were applied.

Three hundred and forty-four samples were examined for the absence of pyrogenic substances. Five samples failed to pass the test.

Tests for the absence of undue toxicity were carried out for 33 samples of Protein Hydrolysate, 18 samples of antibiotics, 4 of Arsphenamine and 6 of Podophyllum. Minimum Lethal Doses in mice for derivatives of P. A. S. and α -Bromo-para-nitro-cinammic aldehyde were determined.

Acute and chronic toxicity tests were undertaken on laboratory animals for three crude indigenous drugs stated to cure leprosy. Each sample was a mixture of 30 to 40 ingredients. On the basis of the experimental data, the therapeutic doses of the drugs were determined.

Out of the 16 samples of Tr. Digitalis, 5 samples were declared to be of non-standard quality. Amongst the 5 "failed" samples, 4 were tested under the Drugs Act.

Examination of drugs by bacteriological and serological methods.—Common antibiotics (Penicillin, Streptomycin, Chloromycetin, Aureomycin etc.) numbering about 80 were analysed during the year under report. Penicillin ointment base, in tablet forms or in lozenges and in combination with Procaine Penicillin constituted the bulk of the total antibiotics tested here. Only one sample was declared to be of non-standard quality.

Eight samples of Dihydrostreptomycin were tested. Two of them failed to comply with the pharmacopoeial specification for 'clarity of solution'. The rest of the samples were of standard quality.

Except one, all the samples of Chloromycetin numbering 14 were found to be of standard quality.

Three samples of Aureomycin were tested, but 2 of them were devoid of antibiotic activity. They contained some inert material and colouring matter.

Seven samples of Antitoxins (Tetanus 5 and Diphtheria 2) were biologically standardised. The potency of all of them conformed to the claim made on the labels except in the case of one "time-expired" sample.

All the solutions or powders meant to be used parenterally were tested for their sterility. Out of 294 such samples, 20 of them failed to comply with sterility test.

VIII. Department of Chemotherapy.

During the year under review, the Department continued its manufacturing and research activities.

Manufacturing activities.—About 2,250 lbs. of sulphathiazole in the form of tablets and injectibles were sold during the year as against 3,946 lbs. in the previous year.

An innovation introduced during the year was the preparation and supply of sodium sulphathiazole injectible in the form of dry powder instead of the aqueous solution as was being done previously, which has the following benefits: (1) longer shelf-life, (2) no discoloration and (3) no reaction with glass container.

About 6,000 lbs. of "Pemon" (antiseptic with R.W. 3.5 to 4) were sold during the year.

The manufacture and supply of drugs for the treatment of Leprosy was continued. About 1,34,250 cc. of Sulphetrone and 160 lbs. of Hydnocarpus oil (injectible) were distributed to the various Leprosy Clinics. In view of very poor demand for the Hydnocarpus oil injectible, the manufacture of this item has been discontinued.

An important consideration relating to the manufacture of Sulphathiazole is the high Import Duty paid by the Institute on the raw materials required for the manufacture of sulpha drugs. The anomalous position can be gauged by the fact that whereas an importer of the finished sulphathiazole or a processor who imports the penultimate product has to pay an Import Duty of only 14 per cent., the Institute, which imports the basic raw materials has to pay a duty of about 38 per cent. Thus, the Institute which alone undertakes the manufacture of sulphathiazole in the true sense of the word (*viz.*, from basic raw materials) is seriously handicapped. The authorities

concerned with the fixation of Import Duties have already been appraised about the situation and it is earnestly hoped that the anomaly will be remedied at an early date by fixing the Import Duty on basic raw materials at a considerably lower level than that of the finished product or penultimate intermediates, or even waiving it off completely so as to encourage the manufacturers in this country to take up the production of synthetic drugs from basic raw materials and thus help the country to be self-sufficient in the matter of life-saving drugs.

The steady increase in the demand for vitamin tablets continued throughout the year. In all, 109, 20,725 vitamin tablets were sold during this year against the last year's figure of 94,99,950. With the introduction of the Employees' State Insurance Scheme, there was a slight additional increase, but this was not as much as was anticipated. The Institute has been making separately Vitamin A-D tablets (oil-soluble vitamins) and B-complex and C tablets (water-soluble vitamins). However, some practitioners do prescribe a single vitamin tablet (multivitamin) which contains both the oil-soluble and the water-soluble vitamins together. Investigation is undertaken to study the manufacture of these tablets. In addition to the manufacture and sale of vitamin products, the department has also tabletted 2,075 lbs. of sulphathiazole and preparation of sulphadiazine and sulphaguanidine tablets is also being taken up soon. Besides, the department has been entrusted with the distribution of Quinine and its substitutes, which is the surplus stock formerly being distributed by the Superintendent of Prohibition and Excise, Bombay. In addition, the department has now been requested to manufacture quinine tablets for the Employees' State Insurance Scheme which is being taken up accordingly.

In view of the expanding activities, and the need for a large number of smaller packings of vitamin tablets, it is felt that a separate Packing Section of the Department with adequate mechanisation for washing and drying of bottles may be required in future.

The following table gives the comparative sales of the various products for the year 1952, 1953 and 1954 :—(Please turn overleaf).

Research Section.—Interesting results in novel synthesis of the pyrimidine and thiazole moieties of Thiamin (Vitamin B₁) have been obtained. Investigations in the chemistry of thiazoles was continued with particular reference to compounds which might possess possible therapeutic value. A large number of new compounds have been synthesised during the year and their structures characterised by analysis. The investigation into their therapeutic value is being undertaken.

Analysis.—Nearly 600 micro-analyses (carbon, hydrogen, nitrogen etc.) were carried out during the year for characterization of the structure of the compounds synthesised by the Research Section.

In addition to the various analyses of sulphathiazole powder as well as tablets, the department also carried out about 350 analyses of various vitamin tablets manufactured by the department. If the demand for vitamin tablets increases materially in future, extra equipment and assistance in the analytical section may become necessary.

Table Indicating the Sale of Vitamin Tablets during the years 1952, 1953 and 1954.

Serial No.	Kind of tablets.	Rate per 1,000 tablets.	Number of tablets sold during the period.		
			1952	1953	1954
1	Vitamin A-D tablets ...	Rs. a. p. 14 0 0	33,14,875	32,91,150	37,37,475
2	Vitamin A-D (Special) ...	20 0 0	88,000	1,32,000	88,000
3	Vitamin A (Special) ...	65 0 0	7,000	6,000	1,000
4	Vitamin B C tablets ...	16 0 0	46,92,700	50,95,350	54,71,100
5	Vitamin B-C ₃ tablets ...	53 0 0	3,16,400	4,82,250	8,68,050
6	Vitamin B ₁ tablets ...	28 0 0	2,09,125	1,47,150	2,06,950
7	Vitamin B ₂ tablets ...	30 0 0	40,175	31,450	65,300
8	Vitamin C tablets ...	28 0 0	2,48,075	2,14,900	3,33,450
9	Acid Nicotinic tablets ...	6 0 0	1,36,650	97,700	1,49,400
10	Vitamin B-C (Special) ...	130 0 0	2,000	Nil.	Nil.
11	Vitamin C (Special) ...	75 0 0	Nil.	2,000	Nil.
Total sale of Vitamin tablets only.			92,55,000	94,99,950	1,09,20,725
Sulphathiazole tablets ...			19,42,500	35,20,600	19,99,550
Total sale of all kinds of tablets.			1,11,97,500	1,30,20,550	1,29,20,275
Total sale-value of Vitamin tablets only.			Rs. a. p. 1,58,362 11 0	Rs. a. p. 1,68,008 7 0	Rs. a. p. 2,05,680 8 0
Total sale-value of Sulphathiazole tablets only.			67,987 8 0	1,23,479 8 0	69,984 4 0
Total sale value of all kinds of tablets.			2,26,350 3 0	2,91,487 15 0	2,75,664 12 0

IX. Department of Biochemistry.

This department has been engaged in active work during the year under review. The various tests carried out on samples received from hospitals, nursing homes and private practitioners numbered 5,903 as against 7,158 last year. The accompanying table gives an idea of the examinations carried out during 1954 as compared to the preceeding four years :—

	1950	1951	1952	1953	1954
Blood Examinations ...	1,684	2,170	2,637	3,752	3,468
Urine ...	194	179	149	280	241
Cerebrospinal Fluid and Body Fluids .	638	1,236	1,764	1,324	1,165
Stool Samples ...	6	3	9	14	9
Special Tests ...	148	207	397	470	346
Milk Samples ...	92	471	1,596	1,318	674
Total ...	2,762	4,266	6,552	7,158	5,903

There was a considerable decrease in the number of milk samples received for examination of fat and total solids. Also, the hemoglobin estimations were taken up by the department of clinical pathology and that helped to relieve the pressure of work in the department to a certain extent. However, there has been an increased demand for the quantitative estimations of serum proteins, blood sugar, urea, cholesterol, calcium, phosphorus and alkaline phosphatase.

The Seamen's Medical Examination Board continued to refer seamen for glucose tolerance tests. These are cases found temporarily unfit to go on sea due to glycosuria. In the course of the year 68 such cases were examined. Due to lack of sufficient staff it has not been possible to examine more patients.

Research Work :—

(1) A simple micro-method for the estimation of potassium in serum is under investigation.

(2) The blood glycogen levels in normal as well as some pathological cases has been taken up for study.

(3) The serum protein fractions particularly with reference to 'true' albumin, alpha, beta and gamma globulins are being studied in cases of cirrhosis of liver.

X. Department of Virus Diseases including Rabies.

The department continued to (1) afford anti-rabic treatment to patients in Northern part of the island of Bombay, (2) prepare anti-rabic vaccine for issue to anti-rabic treatment centres, (3) assist in the diagnosis of rabies in animals, (4) hold stocks and conduct Yellow Fever Inoculations with the assistance of Yellow Fever Diagnostic Unit sanctioned by the Government of India, (5) conduct research in specific virus diseases like influenza, Q fever, Rabies and small-pox.

(a) *Anti-rabic Treatment Centre at this Institute.*—During the year under report 9051 persons applied for anti-rabic treatment; of these, 8,577 completed the full course of treatment and 473 were 'advice cases'. In cases where there was a doubt about the condition of the dog inflicting the bite and the dog was available for observation, patients were given two injections and were advised to keep the dog under observation for 10 days. At the end of the period of observation, if the dog was healthy, no further treatment was given and the case was classified as 'advice case'.

The number of patients who applied for and submitted to treatment at the Haffkine Institute Centre, present interesting data, which is shown below in Table I :—

TABLE I.—*Details of treatment carried out at the treatment centre of this Institute since 1951-54.*

Details.	1951	1952	1953	1954
Number of persons who applied for treatment.	6,489	7,779	8,714	9,051
Advice cases	624	687	985	473
Fully treated	5,865	7,092	7,722	8,577
Discontinued treatment	Nil.	Nil.	7	1
Reported cases of hydrophobia from those treated at the Institute Centre.	Nil.	Nil.	1	Nil.

(b) *Preparation and Supply of Anti-rabic Vaccine.*—Five per cent. phenolised sheep-brain vaccine (Semple) was prepared as usual and issued to various Anti-rabic Centres recognised by the Institute for supply within and outside the Bombay State. Total number of such centres increased from 153 to 163 during the year, out of which 73 were in Bombay State. The vaccine was also supplied to the Veterinary Surgeons within the State and outside for treatment of animals. 9,760 ml. of 20 per cent. canine vaccine was prepared and supplied for prophylactic use in dogs.

The production and supply of vaccine in ml. as well as in the form of treatments, the number of treatment centres, the number of animals used for production have all gone up considerably during the last five years as seen from the following Tables II and IV. The production and supply of Anti-rabic vaccine in millilitres month by month is shown in Table III.

TABLE II.—*Production of Carbolic Anti-rabic Vaccine and Canine Vaccine in ml. from 1950 to 1954.*

Details.	1950	1951	1952	1953	1954
Anti-rabic vaccine in ml.	16,72,840	19,40,215	23,39,740	26,10,160	27,58,160
Canine vaccine in ml. ...	1,060	1,475	3,470	6,185	9,760

TABLE III.—*Production of Anti-rabic vaccine and supplies in ml. during 1954 month by month.*

Month.		Number of ml. vaccine manufactured.	Number of ml. vaccine issued.
January	..	2,05,860	2,43,987
February	..	2,79,880	2,11,808
March	..	3,37,900	2,25,711
April	..	2,96,640	2,96,304
May	..	2,37,480	1,97,502
June	..	2,15,980	2,06,332
July	..	1,81,400	1,82,396
August	..	2,15,060	1,58,574
September	..	2,05,040	1,38,048
October	..	2,13,320	1,76,823
November	..	1,88,480	1,64,932
December	..	1,81,120	2,18,883
Total	..	27,58,160	24,21,300

TABLE IV.—*Details showing the number of treatments sent to all centres and Veterinary Surgeons, reported cases of hydrophobia from Centres, number of sheep used for production of anti-rabic vaccine and number of treatment Centres.*

Details.	1950	1951	1952	1953	1954
Number of treatments sent to all Centres.	11,468	13,432	17,604	19,230	21,555
Number of treatments sent to Veterinary Surgeons.	606	646	827	804	726
Reported cases of hydrophobia ...	1	Nil	6	3	1
Number of sheep used for production of anti-rabic vaccine.	1,040	1,342	1,337	1,545	1,618
Number of treatment Centres ...	129	134	145	153	163

Only one case of hydrophobia was reported from the Civil Surgeon, Raipur, M. P. A boy who was bitten on hand by a rabid dog, completed the anti-rabic treatment for 14 days but developed symptoms four days later and died. Obviously the incubation period was within 20 days of the bite; such cases rarely benefit by the anti-rabic treatment, even though taken in time.

A total of 27,58,160 ml. of Anti-rabic vaccine was manufactured at this Institute and 24,21,300 ml. was supplied to various Anti-rabic centres during the year. The total number of treatments issued during the year was 21,555 :—

Class I	..	..	..	..	1,905
Class II	..	..	..	..	10,998
Class III	..	..	..	..	8,652

Ten additional centres for Anti-rabic treatment were opened during the year taking the total number to 163. They are shown along with the dates of their sanction :—

Serial Number.	Name of the Antirabic Centre.	Date of Sanction.
1	The Municipal Hospital, Dholka ..	16th November 1953.
2	Sevasangh Sarwajanik Hospital, Modasa.	20th February 1954.
3	Kharaghoda Dispensary, Kharaghoda .	11th March 1954.
4	The Jail and Police Hospital, Seoni ..	15th April 1954.
5	The Ulhasnagar Sub-township No. 3, Kalyan.	15th June 1954.
6	The Barkui Hospital, P. O. Parasia District Chhindwada (M. P.).	1st July 1954.
7	The Wankaner Hospital, Wankaner ..	3rd August 1954.
8	The Barsi Borough Municipal Dispensary, Barsi.	15th September 1954.
9	The Main Hospital, Khamgaon ..	15th September 1954.
10	The Khed Municipal Dispensary, Khed (Ratnagiri).	29th September 1954.

(c) *Diagnosis of Rabies in Animals.*—Brain specimens of suspected rabid animals are sent to the department for the diagnosis of rabies and are received from Bombay City and the districts preserved either in formalin or glycerine. Formalin preserved material is suitable for only histological examination. When received in glycerine the diagnosis is usually made by examination of impression smears and by histological methods if necessary. When no negri bodies could be demonstrated by either of these methods they are subjected to a biological test in mice. Out of 63 brain specimens received 44 were sent in glycerine and 19 in formalin. In addition to microscopic and histological examinations the number of biological tests carried out in mice to diagnose rabies was 28.

TABLE V.—*Number of brain specimens received for diagnosis of rabies from Bombay City and outstations during the year 1954.*

—			Total.	Positive.	Negative.	Unfit for examination.
Bombay City ..	..	41	24	17	Nil	
Out-stations ..	..	24	10	14	Nil	
Grand Total ..	..	65	34	31	Nil	

TABLE VI.—*Statement showing the classification of different animals with the results of their brain examination for rabies during 1954.*

Animals.			Total.	Positive.	Negative.	Unfit for examination.
Dog	..	..	47	30	17	..
Buffalo	..	..	5	1	4	..
Bullock	..	..	1	..	1	..
Horse	..	..	2	1	1	..
Cat	..	..	5	1	4	..
Goat	..	..	2	1	1	..
Guinea-pig	..	..	1	..	1	..
Human	..	..	1	..	1	..
Mongoose	..	..	1	..	1	..
Total			65	34	31	Nil

(d) *Researches carried out in the Department.*—Besides stepping up the production of Anti-rabic Vaccine to meet the growing demand, studies were carried on other viruses such as influenza, vaccinia and variola, rabies which are reported separately under Part II.

(e) *Yellow Fever Vaccine and Inoculation.*—The yellow fever inoculation work and the testing of potency of the yellow fever vaccine stocked here on behalf of the Government of India was carried out by the Yellow Fever Diagnostic Unit at this Institute. Several batches of the Rockefeller Foundation Yellow Fever Vaccine and two batches of the Wellcome Yellow Fever Vaccine were tested for potency. Total number of potency tests performed was 10.

Yellow Fever inoculation figures during 1954 are shown in Table VII. The persons inoculated were air or sea passengers from Bombay proceeding to or passing through yellow fever endemic areas. Figures for 1952 and 1953 are given for comparison.

TABLE VII.

<i>Month</i>	<i>No. of persons inoculated</i>		
	<i>1952.</i>	<i>1953.</i>	<i>1954.</i>
January	753	598	675
February	570	818	506
March	462	916	685
April	719	976	556
May	779	1,204	533
June	859	846	498
July	685	1,011	721
August	706	752	450
September	663	635	439
October	785	759	470
November	650	693	716
December	722	891	597
Total ..	8,353	10,099	6,846

Yellow Fever Vaccine stored at this Institute was distributed to the following Yellow Fever Inoculation Centres :—

<i>Centre</i>	<i>Quantity of vaccine supplied in doses</i>	
	<i>1953.</i>	<i>1954.</i>
(1) The Chief Medical Officer, Porbunder.	280	300
(2) The Port Health Officer, Jamnagar.	500	400
(3) The Director of Sanitary and Health Services, Ceylon, Colombo.	2,000	4,000
(4) The Director General of Medical and Health Services, Goa.	900	300
(5) The Delhi State Health Laboratory, New Delhi.	1,000	2,000
(6) The King Institute, Guindy, Madras.	Nil	1,000
(7) The All India Institute of Hygiene and Public Health, Calcutta.	6,000	6,900
(8) The Air Port Health Officer, Santa-cruz.	1,040	1,100
Total ..	11,720	16,000

Potency of Yellow Fever Vaccine.—Several batches of Rockefeller Foundation Yellow Fever Vaccine were repeatedly subjected to mouse potency test, and it was found that most of them had retained the requisite level of potency after 8 years cold storage at 0-4°C.

Post Vaccinal Reactions.—Out of the 6,846 inoculations carried out during the year, no cases of post vaccinal reactions were reported.

XI. Blood Bank Department.

During the year under report the usual activity of collection of blood and lyophilisation of the plasma was carried on. The demand for dried plasma has been low probably on account of free availability of plasma substitute like Dextran and P. V. P. in the market. The investigation on the cases of isoimmunisation and Rh testing referred from various hospitals was also carried out. Sodium sulphathiazole was so long being added to the anticicoagulant solutions as a preservative. With stricter aseptic precautions in collecting blood, it is not now considered necessary to do so.

The demand for lyophilisation of antivenine ampoules was very heavy and during the year, 27,595 ampoules of 10 cc. each were dried as against 10,174 ampoules last year.

Sodium Sulphathiazole solution which was issued in liquid form so long was also supplied in lyophilised form as it was found to be more stable in this form and 4,534 ampoules of 5 cc. each were lyophilised for Chemotherapy Department.

The demand for blood grouping sera showed further increase during the year. 18,400 cc. of blood with high haemagglutination titre was supplied to Diagnostic Department for preparation of this serum. As sufficiently high haemagglutination titre was not found among the usual blood donors this titre was raised by immunising them with saliva and fairly high titred sera were obtained by this procedure.

The following statements show the work carried out during the year under review :

As usual all samples of blood from donors were grouped and kahn tests were performed on them in the department :

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

Centre.	Type of Donor	No. of Donors bled.	Total No. of donors bled.
Haffkine Institute	Paid	1,098	1,471
	Voluntary	7	
	Jail	366	
Indian Red Cross	Paid	851	856
	Voluntary	5	
Other Hospitals		37	37
Grand Total			2,364

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

<i>Product.</i>		<i>Quantity in litres.</i>	<i>Bottles.</i>
Blood		635·825	
Liquid plasma		321·500	
Dried plasma		324·000	1,296

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

<i>Nature.</i>		<i>Quantity in litres.</i>	<i>Bottles.</i>
Sold		191·000	764
Free		117·000	468
Total		308·000	1,232

TABLE IV.—*Showing the results of Kahn Tests carried out during the year.*

Number of samples tested		1,771
Number of negative cases		1,373 (77·6%).
Number of positive cases		398 (22·4%).

TABLE V.—*Showing Blood grouping reports.*

2,143 samples of blood were grouped according to ABO system. The following is their percentage :—

<i>Group.</i>		<i>No. belonging to the group.</i>	<i>Percentage.</i>
AB		204	9·52
B		708	33·03
A		559	26·09
O		672	31·36
Total		2,143	

TABLE VI.—*Showing RH examination carried out.*

Number of individuals tested		532
Number of R negative individuals		41
Percentage of the negative individuals		7·7

XII. Training of Health Personnel.

The 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 research problems or for routine working 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. The following is the list of persons for whom training was given in the various Departments during the year 1954 :—

Serial No.	Name.	Designation and Address.	Department in which trained.	Duration of training.
1	Shri P. M. Jadhav, B.Sc.	Animal House Care-taker, Hindustan Antibiotics Ltd. Pimpri (near Poona).	Animal-House ...	26th December 1953 to 31st July 1954.
2	Shri Mahommad Omar Khan.	W. H. O. Fellowship holder from Afganistan.	Clinical Pathology, Animal-House and Department of Antitoxins and Sera.	31st December 1953 to 20th June 1954.
3	Dr. M. G. Parikh, M.B.B.S.	Medical Officer in-charge Municipal Dispensary, Dholka.	Department of Virus Diseases including Rabies.	15th February 1954 to 24th February 1954.
4	Dr. S. Ananthaswamy.	Director-Designate to Laboratories under Public Health Department of Government of Mysore.	General working and technical procedure of laboratory.	9th February 1954 to 13th March 1954.
5	Dr. Rasiklal H. Shah, L.C.P. & S. (Bom.)	Medical Officer, Shree Seva Sangh Saravjanik Hospital, Modasa via Talod.	Department of Virus Diseases including Rabies.	14th April 1954 to 21st April 1954.
6	Kumari Kamla R. Gurubaxani, B.Sc.	6-C, Kapoorwalla Mansion, 3rd Pali Lane Colaba, Bombay.	Department of Clinical Pathology and Department of Biochemistry.	1st May 1954 to 31st October 1954.
7	Kumari C. D. Chawla, L.C.P. & S.	Lady Doctor, Ulhasnagar Sub-township, Kalyan.	Department of Virus Diseases including Rabies.	29th June 1954 to 5th July 1954.
8	Shri Bansi Mody, M.Sc.	Chemist, Messrs Unichem Laboratories, Bombay.	Department of Pharmacology.	3rd September 1954 onwards for 6 months.
9	Smt. G. N. Gurshahani, BMS. Cl. II.	Medical Officer, G.T. Hospital, Bombay.	Blood Bank Department.	1st November 1954 to 20th November 1954.
10	Dr. P. S. Menon, M.B.B.S.	Deputy Assistant Director-General, Central Research Institute, Kasauli,	Department of Entomology.	8th November 1954 onwards for 4 months.
11	Dr. R. D. Jha	Secretary Rakta Dana Samiti, Nagpur.	Blood Bank. ...	18th December 1954 to 31st December 1954.

XIII. Critics Club.

Under the auspices of this Club 30 meetings were held during the year. The meetings were held every fortnight. Original research work, reviews and a few abstracts were presented during the meetings by the various departments of this Institute. Two special lectures by guest speakers were arranged during the year. Prof. E. J. Wayne spoke on "Chemotherapy of Bacterial Infections". Dr. Norman Jolliffe, Director, Bureau of Nutrition, Department of Health, New York City, spoke on "Recent Advance in Nutrition of Public Health Significance".

XIV. Miscellaneous.

Apart from the researches carried out by the members of the staff, the following scientific enquiries sponsored by the Indian Council of Medical Research were also conducted during the year 1954 :—

- | | |
|--|--|
| (1) Plague Researches up to 31st March 1954. | Under the Director, Haffkine Institute, Bombay. |
| (2) Enquiry on the Pulicidal Value of Pyrethrums, from 1st April 1954. | Under the Director, Haffkine Institute, Bombay. |
| (3) Enquiry on the Role of Nutritional Factors in Hepatic Cirrhosis. | Under Dr. M. V. Radhakrishna Rao, Assistant Director, Department of Nutrition, Haffkine Institute, Bombay. |
| (4) Enquiry on the Study of Indian Poisonous Snakes. | Under Dr. P. J. Deoras, Assistant Director, Department of Entomology, Haffkine Institute, Bombay. |

In addition to abovementioned scientific enquiries conducted here, the following research fellowships and scholarships were attached to this Institute during the year 1954. Particulars of the sponsors, the subject of fellowship and the candidates working under them are as under :—

Serial No.	Sponsors.	Subject of Fellowship.	Candidates working on the Fellowship.
1	Unichem Laboratories, Bombay.	Researches on "Casein Hydrolysate"	Dr. K. C. Thadani, B.Sc., Ph.D. up to 31st August 1954. Shri J. R. E. Valadares, M.Sc. from 3rd September 1954.
2	Unichem Laboratories, Bombay.	Researches on "Indigenous Drugs."	Shri V. J. Dalvi, M.Sc.
3	Reptakos Medical Research Board, Bombay.	Isolation and Study of biological properties of the therapeutically important constituents of Cobra and Russell's viper venom.	Smt. Shanta S. Rao, M.Sc., Ph.D.
4	Tata Industries Ltd., Department of Industrial Health, Bombay.	Entomological Survey of Kurla and its Environments.	Shri D. R. Ranade, M.Sc., up to 13th August 1954 (A.N.). Shri K. V. Tonpi, B.Sc. from 16th August 1954.

Besides these, there were two other Government Research Scholarships attached to this Institute and they were held by (1) Kumari K. M. Sethi, B.Sc., up to 31st July 1954 and Smt. Shanta V. Agashe, M.Sc. from 18th November 1954 and (2) Shri M. S. Gokhale, M.Sc.

The facilities were also offered to science graduates to take their post-graduate degrees by research at this Institute. During the year 1954, the following candidates were given such facilities under this scheme :—

- (1) Shri K. V. Tonpi, B.Sc. (completed 11th May 1954).
- (2) Shri P. Y. Dighe, B.Sc. (completed 3rd October 1954).
- (3) Shri N. V. Bhagavan, B.Sc. (completed 3rd June 1954).
- (4) Shri S. M. Vaidya, B.Sc. (completed 17th November 1954).
- (5) Kumari Ranjan D. Mehta, B.Sc. (joined on 4th January 1954).
- (6) Shri Yegendra I. Shethna, B.Sc. (joined 3rd September 1954).
- (7) Kumari M. S. Savant, B.Sc. (joined 3rd September 1954).
- (8) Shri J. K. Parikh, B.Sc. (joined 4th September 1954 and discontinued from 3rd December 1954).

XV. Income and Expenditure 1953-54.

Receipts.		Expenditure.	
XXVII— <i>Medical Bacteriological Laboratories—</i>		38— <i>Medical—</i>	
	Rs.		
Sale proceeds of Sera and Vaccines.	26,69,945	I. Bacteriological Laboratories.	
Other Miscellaneous Receipts	51,740	I.—1 <i>Haffkine Institute.</i>	Rs.
		Pay of Officers ...	2,07,005
		Pay of Establishment ...	4,35,347
		Allowances ...	3,31,945
		Contingencies ...	12,51,165
		Research Scholarships ...	2,476
		I.—2 <i>Pasteur Institute.</i>	
		Pay of Officers ...	11,565
		Allowances ...	4,276
	27,21,685		22,43,779

PART II.

RESEARCH WORK.

I. Antigenic Structure of Plague Bacillus and its Relation to Immunity

(WAGLE AND HABBU).

It is known that the plague bacillus has two types of antigen (1) the so-called capsular antigen and (2) somatic antigen. Schutze has claimed that the capsular antigen is necessary for giving adequate protection and therefore recommended that the plague vaccine should be incubated at temperature of 37°C. Formerly the Institute was preparing plague vaccine by incubating at 28°C at which temperature the capsular antigen is not supposed to develop to any appreciable extent. At present plague vaccine is being prepared at 32°C; at this temperature there is an appreciable development of the capsular antigen.

The question however remained as to what role the somatic antigen played in affording immunity against plague infection, and if it did not afford any protection, then whether it would not be enough to prepare vaccine from capsular antigen alone. With this object in view investigation was undertaken. Capsular antigens prepared, from capsulated virulent strain, in different ways were tested in white mice to assess the degree of protection they gave in that animal. The question about the heat liability of the antigens was also studied by boiling the vaccine for different periods. For studying the part played by somatic antigen in giving immunity against plague, avirulent non-protective non-capsulated strain TRU was used. Vaccine prepared from it was tested in white mice. The results obtained are given in the table below :

It would be seen from these results that capsular antigen gives adequate protection and that a very large proportion of capsular antigen is destroyed when vaccine made from virulent capsulated strain is boiled for $\frac{1}{2}$ to 1 hour. There are indications to suggest that the capsular antigen is not totally destroyed by such heating and investigation is being carried out to find out whether there is in the capsular antigen another antigen which is heat-stable. The results also show that the somatic antigen does not give protection ; so far as can be judged from these results it appears that capsular antigen would alone be sufficient to immunise patients against plague infection and probably no part is played by somatic antigen.

Further work is necessary to confirm these results and also to study the components of the capsular and somatic antigens with a view to decide finally the composition of future plague vaccine.

TABLE.

Strains used : P. pestis 195/P
(Virulent : protective).

Strain used : P. pestis T.R.U.
(Avirulent : non-protective).

Vaccine.	Mouse Protective Dose.	Vaccine.	Mouse Protective Dose.
Vaccine killed with P. M. N. only.	0·0048 c.c.	Killed with P. M. N. only.	No protection upto at dose of 0·4 ml.
Ame's antigen extracted with potassium sulphocynide.	0·009 c.c.	Ame's antigen extracted with potassium sulphocynide.	Do.
Schutze's antigen heated at 60°C 1½ hours.	0·027 c.c.	Schutze's antigen Heated at 60°C 1½ hours.	Do.
Culture boiled for ½ hour	... 0·10 c.c.	Culture boiled for ½ hour.	Do.
Culture boiled for 1 hour	... 0·13 c.c.	Live Vaccine.	Do.
Culture boiled for 2 hours	... 0·055 c.c.		

II. Eijkman's Test.

(NIMBKAR, RAJADHYAKSHA AND HAZRA).

Eijkman's test as reported last year is used for distinguishing the Bact Coli Type I (faecal) from rest of the coliform organisms of non-intestinal origin. This test depends upon the capability of Bact Coli Type I to produce acid and gas in MacConkey broth or in a similar medium at 44°C; the organisms of *Aerogenes* Intermediate Cloacae subgroups being unable to ferment lactose at this temperature.

For the successful application of the test rigid temperature control in the medium in which the organisms is growing is necessary. This was achieved in our laboratory by utilising a double walled Kostansky bath fitted with a sensitive thermostatic control. The top of the bath was then covered with an asbestos cement lid and an efficient electric stirrer was used to stir the water in the bath. The temperature of the medium was recorded by introducing thermometer in a tube of MacConkey broth. This improvised apparatus was found very satisfactory, maintaining the required temperature at 44°C constantly $\pm 0.5^{\circ}\text{C}$.

The results of 13 strains were reported last year. In continuation of the same 45 more strains were subjected to this test. These strains were isolated during routine water examination for suitability for drinking purposes.

Out of these 10 were typed as Bact Coli Type I, 18 *Aerogenes* Type I, 3 Intermediate Type I, 2 Intermediate Type II and twelve irregular according to their I.M. Vi. C. test results.

All the 10 Bact Coli Type I (faecal) strains showed acid and gas production within 18 hours at 44°C.

Out of 18 strains of Bact *Aerogenes* Type I tested, 17 were negative for 48 hours. Only one strain which was negative upto 18 hours, showed a positive test after 24 hours at this temperature.

Three strains of Intermediate Type I were negative for 48 hours for production acid and gas at 44°C.

Out of two strains of Intermediate Type II, one showed a positive test within 18 hours while the other remained negative till 48 hours at 44°C.

Out of the twelve irregular strains subjected to the test, five were negative after 48 hours. But six showed a positive reaction within 18 hours while one which was negative upto 18 hours showed a positive test after 24 hours. All these 45 strains tested produced acid and gas within 24-36 hours at 37°C.

The results of 58 strains of coliform group tested so far show that Eijkman's test is useful in case of majority of the coliform strains, isolated mainly from water from round about the Bombay City, excluding those of the irregular type, for distinguishing the bact coli Type I (faecal) from those of *Aerogenes* Cloacae group.

Further work is in progress.

III. Preliminary Studies on Immunity Response in mice with mixed Antigens (Bacterial Vaccines and Toxoids).

(HAZRA AND HABBU)

It has been reported by various authors that when a mixture of antigens is administered to animals, the resulting response to the individual antigens is reduced to a lower level than when it was administered individually. Scheibel (1944) showed that the response in guineapigs to a mixture of diphtheria and tetanus toxoids was lower to both the components than when they were injected alone. Whereas, there are other workers who claim a synergic effect when combined antigens are administered, Ramon and Richou (1950) working with mixtures of TAB Vaccine and tetanus toxoid showed that a much higher tetanus anti-toxin titre was obtained than the control group injected with tetanus toxoid alone.

In view of the very conflicting reports on the subject we started our experiment to study the response to TAB Vaccine in mice when administered along with tetanus toxoid. We immunised groups of mice with varying doses of a mixture of TAB Vaccine and tetanus toxoid and challenged them with mucinised suspensions of *B. typhosus*. We also included in the study mixtures of the antigens in different proportions, as this is claimed to be one of the conditions to get synergic effect. The preliminary results we have so far obtained indicate that the response to TAB Vaccine is independent of the presence of tetanus toxoid. The response to Tetanus immunization was satisfactory. Further work is in progress. Work is also in progress to study the effect of bacterial antigens when administered in combination with Diphtheria and Tetanus Toxoids.

IV. Purification of Antitoxin by Enzyme Digestion Treatment.

(MORE AND RAO)

Preliminary experiments on pepsin digestion of antitoxic plasma gave 30 to 50 per cent. purer product but the yields were low. Work was continued this year with an idea of increasing the yields as far as possible. Enzyme digestion of the plasma proteins was carried out under different conditions of pH and temperature using processed and unprocessed sera. It was observed, however, that digestion if carried out with globulin solution concentrated by ammonium sulphate according to the method given below resulted in increasing the total yields and also getting purer products.

The method adopted was essentially that of Pope but slightly modified as follows:—Antitoxic serum fractionated with ammonium sulphate was diluted to protein concentration of 3 to 4 per cent. 0.1 per cent. Pepsin was added and pH of the solution adjusted to 4.0 and the bottles containing the solution were kept at 37°C for 24 hours. 150 gms. of powdered ammonium sulphate per 1000 ml. was added and the reaction was adjusted to pH 4.2. The solution was then heated on a water bath at 58°C for half an hour, allowed to cool and filtered. The filtrate which contained the degraded antitoxins was neutralised and saturation of ammonium sulphate was increased to 50 per cent. The precipitated antitoxin was filtered, pressed and dialysed in distilled water.

Antitoxin titres of the dialysed solution were determined by flocculation test. In case of five experimental lots the purity was found to have increased nearly two-fold. The final recovery of unitage obtained was as high as 79 per cent.

Electrophoretic analysis of enzyme treated serum by paper electrophoretic instrument gave a single zone, which had least electrophoretic mobility. This probably indicated the removal of alpha- and beta-globulin, giving gamma globulin in the purified fraction.

V. Activity of Different Fractions of Rabbit and Horse Antivenin Sera.

(KULKARNI AND RAO)

100 c.c. of rabbit anti-cobra-venom serum and horse polyvalent antivenin serum were first fractionated into globulin fraction (insoluble in 50 per cent. saturation of ammonium sulphate) and Albumin fraction. The globulin fraction was dialysed and separated into eu-globulin fraction (water insoluble) and Pseudo-globulin fraction (water soluble). All the fractions were dialysed and dissolved in normal saline. The solutions were tested for activity by flocculation test as well as by neutralization test with cobra venom. Over 80 per cent. of activity was found in Pseudo-globulin fraction in both rabbit and horse sera. Less than 10 per cent. activity was associated with eu-globulin and Albumin fractions of rabbit and horse sera.

VI. Proteases in Cobra and Russell's Viper Venoms.

(SHRIMATI SHANTA RAO)

The proteolytic activity of dialyzed cobra (*Naja naja*) and Russell's viper (*Vipera russelli*) venoms was determined at short intervals of pH between 1 and 10.5 according to Anson's procedure using 2 per cent. heat-denatured haemoglobin as substrate. A universal buffer consisting of barbiturate-phosphate-acetate was used. Five peak values were observed at pH's 1.0, 3.0, 5.1, 6.5 and 9.2 in cobra venom suggesting the presence of 5 different proteolytic enzymes in it. Russell's viper venom had two powerful proteolytic enzymes one active at pH 4.0 and the other at pH 9.0.

VII. Common Antigens in Pasteurella.

(BHAGAVAN, NIMBKAR AND RAO)

Common antigens between *P. pestis* and *P. pseudotuberculosis* were determined by the previous workers like Schutze, Bhatnagar and Seal, using serological precipitin and agglutination techniques. They have reported the presence of one common antigen between them. But these procedures suffer from the disadvantage that it is difficult to detect more than one common antigen by these techniques.

We determined the common antigens using the recently developed Oudin's technique, and it was found that there are as many as four common antigens between Virulent and Non-virulent strains of *P. pestis* and also equal number between *P. pestis* and *P. pseudotuberculosis*.

We also determined the common antigens between the *P. pestis* vaccine heated at 100°C for $\frac{1}{2}$ hr. and *P. pseudotuberculosis* vaccine and it was found there is one common antigen. This antigen may be the heatstable "somatic" antigen of *P. pestis* of Schutze.

VIII. Extraction of Antigens of *P. pestis* using Ethylene Glycol and Diethylene Glycol.

(BHAGAVAN AND RAO)

The crude and dry antigenic material obtained from plague vaccine by adding ammonium sulphate crystals to saturation was extracted with organic solvents, ethylene glycol and diethylene glycol, according to the methods of Morgan and co-workers.

The dry powder was repeatedly extracted first with ethylene glycol and this fraction was named as Fraction A. The residue was again repeatedly extracted with diethylene glycol and the Fraction was named B. The final residue was extracted with Normal saline and the extract was named as Fraction C.

These dialysed fraction A, B and C were tested for their homogeneity by Oudin's test, and it was found all the extracts contained one antigen in very large proportion and two in traces. The fractions were also tested for their antigenicity in mice by the method of Sokhey against a challenge of dose 1600 virulent organisms (160 m.l.d.). The fractions A and B were found to be highly immunogenic and their 50 per cent. end points were 8 and 8.83 micrograms respectively. The fraction C was found to be non-protective indicating that it corresponds to "Somatic" antigen of Schutze.

IX. Sex Ratio and Specificity of some Fleas on Bombay Rats.

(DEORAS TONPI AND GOKHALE)

The fleas collected in 1953 on *Rattus rattus* and *Bandicota bengalensis* (Gunomys kok) showed the following percentage of sex.

X. cheopis 57.2 per cent. males and 42.9 per cent. females.

X. astia 31.2 per cent. males and 68.8 per cent. females.

The majority of *X. cheopis* were found on *R. rattus* and *X. astia* on *B. bengalensis*. Further collections during this year have shown as in Table I that *X. cheopis* is predominant on *Rattus rattus*. These observations were compared with those of Webster and Chitre in 1930, as given in Table II and III.

It will be noticed that our observations are similar so far as sex ratio of these fleas and the specificity of them on *R. rattus* and *B. bengalensis*. It is interesting to note that while Webster and Chitre collected the fleas from rat traps covered by a canvas bag, while we did not use any bag. Observations are further being done in the field to confirm whether a canvas bag is necessary for flea surveys.

From Table III one could find that though the percentage of *X. astia* is higher on *B. bengalensis* during Webster and Chitre's as well as our observations, a distinct increase on the incidence of *X. cheopis* on this rat has been noted by us.

From the collection of rats coming to this Institute one finds a gradual increase in the number of *B. bengalensis*. Since last ten years, *R. rattus* has been found to be getting resistant to plague infection while *B. bengalensis* is very susceptible. *X. cheopis* has been shown to be the best transmitter of plague, and thus an increase in the number of a susceptible species of rat harbouring a gradual increasing number of plague transmitting fleas needs careful study from the point of view of plague epidemiology in Bombay town.

TABLE I.—*Distribution of fleas on Rats.*

	<i>R. rattus.</i>	<i>B. bengalensis.</i>	<i>R. norvegicus.</i>	<i>B. malabarica.</i>	<i>M. musculus.</i>	<i>S. caeruleus.</i>
<i>X. cheopis</i> ...	107	21	21	5	3	2
<i>X. astia</i> ...	19	11	77	4	5	

TABLE II.—*Percentage of fleas in the collections.*

	<i>X. cheopis.</i>			<i>X. astia.</i>			Remarks.
	Total.	Male.	Female.	Total	Male.	Female.	
Webster and Chitre 1930.	69·6	62·2	39·8	27	39·0	61·0	
Present studies 1953-54.	76·3	57·2	42·8	23·7	31·2	68·8	

TABLE III.—*Percentage of fleas on rats showing specificity.*

	<i>R. rattus.</i>			<i>B. bengalensis.</i>		
	<i>X. cheopis.</i>	<i>X. astia.</i>	<i>X. brassiliensis.</i>	<i>X. cheopis.</i>	<i>X. astia.</i>	<i>X. brassiliensis.</i>
Webster and Chitre ...	83·6	11·7	4·6	22·8	76·1	0·7
Present studies ...	89·04	10·96	0	58·8	41·2	0

X. Venom Tolerance in Russell's Viper.

(DEORAS AND MASHALKAR).

It has been observed in a number of experiments that 0·25 c.c. of Russell's viper serum neutralised nearly 2 M.L.D. of Russell's viper venom (0·5 mgm. of *R. viper* venom). On this basis a normal Russell's viper snake with about 100 c.c. of the total blood may tolerate 85 mgm. of Russell's viper venom.

Further work on Russell's viper serum has shown that this completely inhibited the anti-lecithinase activity of Russell's viper venom when tested on rabbit blood. This serum also shows a trypsin inhibiting activity which is about $\frac{2}{3}$ th that of human serum.

XI. The Influence of Snake Venom and Sera on Rabbit Erythrocytes.

(DEORAS AND MASHALKAR).

It has been previously noted by a number of workers that the Indian Cobra venom would cause haemolysis of the red blood corpuscles of certain mammals but the serum of this snake inhibited this haemolysis.

We have tested this on rabbit blood. This was centrifuged and the blood cells washed in saline. These cells were then diluted to the original volume of the blood and the concentration of venom added on a M.L.D. basis in the following arrangements. 0·4 c.c. of Cobra, Russell's viper and Krait Venom contained 0·026, 0·08 and 0·013 mgms. of the venom respectively :—

Tube 1—0·4 c.c. venom, 0·1 c.c. saline, 0·3 c.c. washed blood cells.

Tube 2—0·4 c.c. venom, 0·1 c.c. serum, 0·3 c.c. washed blood cells.

Tube 3—0·4 c.c. saline, 0·1 c.c. serum, 0·3 c.c. washed blood cells.

The tubes were kept at 37°C. for 24 hours. The action was judged by the change of the opaque red to translucent straw colour and confirmed by the disappearance of red cells microscopically. The results are given below :—

<i>Venom.</i>		<i>Serum.</i>		<i>Result.</i>
R. viper	..	R. viper	..	Complete inhibition.
R. viper	..	Cobra	..	Partial inhibition.
R. viper	..	Krait	..	Do.
Cobra	..	Cobra	..	Do.
Cobra	..	R. viper	..	No inhibition.
Cobra	..	Krait	..	Do.
Krait	..	Cobra	..	Do.
Krait	..	Krait	..	Partial inhibition.

XII. Ratex as a Rodenticide.

(DEORAS)

This product was submitted for trial on *Rattus rattus*, *Bandicota bengalensis*, and *Rattus norvegicus*. The trial was done on a comparative basis with Barium Carbonate. Poison was administered in stipulated doses with Bajra flour, and the baits prepared were kept for a period of 17 hours at a time. The following table represents the efficacy of these two rat poisons.

The percentage of Rat Mortality.

Name of rats.		Percentage mortality with Ratex.	Percentage mortality with Barium Carbonate.	Remarks.
<i>Rattus rattus</i> (House rat)	...	16.7%	83.3%	<i>Rattus rattus</i> showed disinclination to eat Ratex bait. In spite of complete pills being swallowed a few of these rats did not die.
<i>Bandicota bengalensis</i> (Field rat)	...	83.3%	100%	Ratex pills became spongy during rains and very hard during dry spells within 17 hours.
<i>Rattus norvegicus</i> (Drain rat)	...	25%	100%	

XIII. Immunity Response to Semple's Anti-rabic Vaccine.

(DOGRA).

This study, started in 1951, was concluded during the year under report. Data was presented in last year's report to show that by using the mouse protection test technique sera from persons treated with Class II and III treatments showed presence of viricidal bodies for 9-12 months after treatment. Also that during early stages of immunisation there was no difference whether one gave class II or III treatment.

Pooled sera from patients treated with Class II or III treatments were taken and subjected to quantitative test. The results are tabulated below :—

Table showing results of mouse protection test on pooled sera collected from Class II and III cases—14 days after starting the treatment.

Pooled sera class.		1 : 4	1 : 8	1 : 16	1 : 50	1 : 250	1 : 500
Class II	...	2/5	1/5	1/5	0/5	0/5	0/5
Class III	...	5/5	4/5	3/5	0/5	0/5	0/5

Test virus used was in a dilution of 10^{-4} freeze-dried mouse fixed pool VIII.

It would be observed that on quantitative estimation of rabicidal bodies present in pooled sera of cases treated with Class II and III treatments there appeared no material difference and that as early as 14 days after starting treatment with Semple's vaccine rabicidal bodies were detectable in 1 in 16 dilutions of human sera of cases of both Class II and III.

ADVICE CASES.

It has been a practice in this Institute to consider cases in which the biting animal is alive and well and available for observation as "advice cases". These cases are assigned the class according to Hempt's classification and given two doses of anti-rabic vaccine appropriate to the class to which assigned. The biting animal is observed and if alive and well for 10 days, no further treatment is given. In the event of the death of the animal due to Rabies, appropriate treatment is started immediately and a full course of treatment is given.

The degree of immunity confirmed by this procedure was put to test. The neutralisation mouse test was performed with sera of patients classes as "advice cases". The sera were obtained before treatment and ten days afterwards. The results are set out in the following table:—

Table showing results of mouse protection test with sera from "Advice Cases".

Serial No.	Patient's No.	Date of treatment.	Class of treatment.	Results.		Remarks.	
				1st sample.	2nd sample.		
1	5287	30th July 1953	...	II	...	5/5	1st sample collected before treatment.
2	5320	31st July 1953	...	II	...	0/5	
3	6221	17th September 1953	...	II	0/5	3/5	
4	6384	24th September 1953	...	III	0/5	2/5	2nd sample collected ten days after starting treatment.
5	6420	27th September 1953]	...	II	0/5	2/5	
6	6424	27th September 1953	...	III	0/5	2/5	
7	6545	3rd October 1953	...	III	0/5	0/5	
8	6561	4th October 1953	...	III	0/5	3/5	

Note.—Class II advice cases received 5 c.c. twice and Class III 10 c.c. twice.

It would appear that the production of rabicidal bodies in man with two injections of 5 c.c. and 10 c.c. each, varied from individual to individual and appeared very little, but showed no difference between Classes II and III. None of the sera obtained prior to administration of the vaccine showed presence of rabicidal bodies.

XIV. A Lyophilised Anti-rabic Vaccine.

(DOGRA).

During the courses of studies on Antirabic Vaccine it was demonstrated that Semple's vaccines deteriorated in time even when stored at $\pm 5^{\circ}$ C and rapidly deteriorated if stored at room temperature. In India facilities for cold storage are few and far between. It was, therefore, considered that a lyophilised preparation not requiring cold storage would be most suited, provided it had an adequate degree of potency.

From 1951 onwards attempts were made to prepare such a product. Four batches were made and tested with Habel's technique. Simultaneously was tested Semple's Vaccine prepared from *same* sheep's brain. Semple's Vaccine was made in the usual way. Lyophilised Rabies Vaccine was prepared by milling brain substance with chemically pure chloroform (for inactivation of

the virus) and freeze drying in Cryochem apparatus and sealing in vacuum. Moisture content was determined by phosphoric pentoxide method. The results giving potency values of this vaccine are tabulated below :—

Table showing method of preparation, viability test and potency values of lyophilised and Semple's vaccine prepared from the same sheep's brain.

Serial No.	Batch No. and date of preparation.	Method of preparation.	Viability Test.	Moisture Content.	Results of Habel's Potency Tests.	
					1-5 weeks old.	12 months old.
1	704/51	(1/2 Brain Semple's Vaccine ;	...	...	19,050	12,590
	2-6-51	1/2 Brain 1:3 Gumacacia and 20 minutes milling with 1.5 chloroform.	0/10	1.18% (23-4-54)	58,880	35,480
2	811/51	(1/2 Brain Semple's Vaccine ;	...	...	..	2,570
	27-6-51	1/2 Brain 1:3 Gumacacia and 20 minutes milling with 1.5 chloroform.)	0/10	1.75% (24-4-54)	..	10,000
3	880/51	(1/2 Brain Semple's Vaccine ;	...	...	32,360	...
		1/2 Brain 1:3 Gumacacia and 20 minutes milling with 2% chloroform).	0/10	...	70,790	...
4	898/51	(1/2 Brain Semple's Vaccine ;	...	...	...	7,943
	25-7-51	1/2 Brain 1:3 Gumacacia and 20 minutes milling with 1.5 chloroform).	0/10	1.00% (25-7-51)	...	22,390

It would be observed that (a) all the four samples of lyophilised vaccines showed no evidence of live virus, (b) all the samples showed slightly better potency value than Semple's vaccine prepared from the one half of the same sheep brain, (c) even months later whereas Semple's vaccine showed very low potency values the lyophilised vaccine showed satisfactory potency levels as per accepted standards namely 50 per cent. protection against 1000 m.l.d. of virus, (d) moisture content varied from 1 to 1.75 per cent. almost three years after the vaccine had been prepared.

XV. A Lyophilised Canine Vaccine.

(DOGRA).

When rabies vaccine was successfully lyophilised and found to possess high degree of potency it was tested in mice giving a 20 per cent. brain suspension in a single shot in a way similar to the method using for testing Phenol-killed 20 per cent. single shot Canine Vaccine. The results are set out in the following table :—

Table showing Potency Values of Lyophilised Canine Vaccine.

Serial No.	Batch No. and Date of Preparation.	Age of vaccine at the time of test.	Potency Value.
1	638/51	...	...
	23-5-51	3 months.	70,790
2	655/51	...	...
	24-5-51	3 months	31,700
3	704/51	...	...
	1-6-51	2 months	7,096

It will be observed that lyophilised Canine Vaccine which is a 20 per cent. sheep brain, milled chloroform-killed and lyophilised vaccine when assayed in mice showed a very high degree of potency. The usual 20 per cent. sheep brain phenol-killed Canine Vaccine prepared by the author gave potency values (12 determinations) between 7,244 and 70,790. The lyophilised product compared very favourably with the carbolised Canine Rabies Vaccine.

XVI. Chloromycetin in Typhoid Fever.

(DOGRA AND MODI).

Investigations in connection with effects of Chloromycetin in culturally positive enteric group of fever cases was concluded during the year under review. In all, 100 culturally positive cases were studied by repeated blood culture, triple Widal Test, urine culture and stool culture at regular intervals. Synthomycetin, Kemycetin and Chloromycetin showed no difference in their clinical effects.

Bacteraemia disappeared in 3-4 days after treatment with this group of antibiotics. Triple Widal Tests showed no rise of titre.

XVII. Pregnancy Test.

(DOGRA AND MODI).

In order to establish the validity of the Rabbit and Rat Tests for pregnancy the follow-up scheme started in 1952 was continued and concluded during the year under review. An analysis of 1,000 followed up cases showed that the 48 hours Rabbit Test gave true results in 96.9 per cent. of cases; the 24 Hours Rat Test 99.5 per cent. and the 3 Hours Rapid Rat Test in 97.4 per cent. of cases. These results conclusively show the reliability of the 24 Hours Rat and the Rapid Rat Tests and suggest that for every sample the Rapid Rat Test should be carried out along with a 24 Hours Rat Test. In urgent cases Rapid Test will help the clinician to decide upon the line of treatment without delay.

XVIII. Anaerobically Grown Typhoid Organisms in Widal Test.

(NARASIMHAM AND SHAIKH).

Further agglutination studies with emulsions made of anaerobically grown strain of *S. typhi* 0-901, were carried out along with standard emulsions used for routine Widal Tests. The results show that the anaerobically grown culture suspension has an increased agglutinability and clarity of reaction.

Comparative studies with phenol-killed and formalin-killed (anaerobic culture) emulsions were also carried out. The results indicate that phenol-killed emulsion has a higher clarity of reaction while formalin-killed emulsion gives rise to a higher titre of reaction.

Further studies are in progress.

XIX. Tropical Eosinophilia.

(An experimental study on Laboratory animals.

(KOLAH AND MODI).

Further work on experimental observation of laboratory animals after inoculation of material such as blood, sputum, throat swab cultures, etc., from cases of suspected Tropical Eosinophilia was continued with the collaboration of Dr. B. D. Patel, Hon. Pediatrician, B. J. Hospital, Bombay. The period of keeping the experimental animals under observation was extended to 2-3 months as it was found that the period of 2-3 weeks was not sufficient for complete reaction to be manifested.

Further work along these lines is in progress.

XX. Use of Dyes in Serodiagnostic Tests for Syphilis.

(SAMANT)

(i) *Sensitizing action of dyes on Meinecke's Antigen.*—Further work on use of dyes as sensitizers of the Meinecke and V. D. R. L. Antigens was continued. The encouraging results reported earlier with Brilliant green and other dyes on Kahn Antigen, suggested to us the use of the dyes for enhancing the sensitivity of Meinecke Antigen. Meinecke has used only small amounts of Victoria blue 4R in presence of tolu balsam, but the results with this dye were not very encouraging. Larger quantities of Victoria blue 4R and other were used in our series of experiments with a view to test their sensitizing and indicator action on Meinecke Antigen.

Eight dyes, viz. Ecsin, Scarlet red, Janus green, Thiomin, Victoria blue 4R, Brilliant green, Acriflavine and Methyl blue were studied for their sensitizing action on the Meinecke Antigen. About 500 sera were tested with each dye by Meinecke micro slide test. Four dyes, viz. Victoria blue 4R, Brilliant green, Acriflavine and Methyl blue were found to be good sensitizers and Victoria blue 4R was found to be the best. In absence of tolu balsam, the dyes did not show any sensitizing action. The Meinecke Antigen, sensitized with tolu balsam and Victoria blue 4R showed sensitivity better than the routine Meinecke micro slide test antigen and this compared favourably with V. D. R. L. Test.

(ii) *Sensitizing action of dyes on V. D. R. L. Cardiolipin Antigen.*—The possibility of increasing the sensitivity of the V. D. R. L. Antigen by use of dyes was investigated. The three dyes, viz. Victoria blue 4R, Janus green and Brilliant green was tried. These dyes were chosen because of their proved sensitizing action on Kahn and Meinecke Antigens. No resin or gum was used as this would be lowering the purity of cardiolipin in the V. D. R. L. Antigen.

Of the three dyes studied, only Brilliant green showed some sensitizing action when added to the alcoholic solution of the antigen and also when a solution of the dye in distilled water or saline was added to the suspension of the V. D. R. L. Antigen.

The increase in the sensitivity did not affect the specificity of the test, and the dye acted better in the lower saline concentration than in the higher one.

XXI. Studies on Cardiolipin-Type of Phospholipids and their Use in the Serodiagnosis of Syphilis.

(SAMANT).

(i) *Presence of Cardiolipin-type of phospholipids in different animal tissues.*—Cardiolipin is generally prepared from beef heart, however, it has been suggested by Pangborn that it would be possible to prepare cardiolipin-type of phospholipids from other animal tissues also. The present investigation was undertaken with a view to ascertain the existence or non-existence of cardiolipin-type of phospholipid in different organs of mammals.

Tissue extracts of heart, liver, kidney and muscles of ox, sheep, horse, rabbit and cat were prepared using alcohol-ether mixture after preliminary treatment with acetone. The extracts were tested for their serological activity and lipid constituents. The amount of cholesterol, total phospholipid, lecithin and cardiolipin-type of phospholipid in the tissue extracts were estimated. Extracts were also prepared from four commonly occurring fishes, viz. Bombil, Collumbi, Bangara and Manjyali, and analysed as above. All the extracts were tested by V. D. R. L. slide test technique with known positive and negative sera and the results showed definite serological activity towards syphilitic sera. The presence of cardiolipin-type of phospholipid was established by chemical analysis. The liver and kidney extracts from all the animals showed maximum amount of cardiolipin-type of phospholipids.

(ii) *Isolation and purification of cardiolipin-type of phospholipids from beef liver and beef kidney.*—Beef liver and kidney were found to contain maximum amount of cardiolipin-type of phospholipid and were therefore used for the preparation of the cardiolipin-type of phospholipid. The isolation and purification was carried out by the method of Pangborn as described in the W. H. O. monograph on cardiolipin with slight changes to suit our laboratory conditions.

The yield of the phospholipid varied in different experiments from 10-18 mg. per 100 g. of the starting material both in the case of beef liver and kidney. The finally purified phospholipid was used as antigen in the V. D. R. L. test and the results compared well with standard V. D. R. L. cardiolipin antigen. It was analysed for phosphorus percentage, iodine number etc., and results obtained were found to compare favourably with the values given in literature. The phospholipids thus isolated showed very good resemblance to cardiolipin.

XXII. Studies on Influenza Virus.

(SOMAN AND DIGHE).

(a) *Isolation of Virus Strains.*—During the year, isolation of Influenza virus from stray and sporadic cases was continued as before. Fifteen throat washings were tested in eggs and five haemagglutinating virus strains were isolated during the months of May-June and July. Since May, a small localised outbreak of sore throat and cold was reported to be around Dadar and Parel area of this city. Out of these five strains, three were recovered from Institute personnel and two from a Doctor's family. These strains were identified as those of Influenza belonging to Type A, Liverpool Sub-type, by us and were further confirmed by the World Influenza Centre, London.

It was reported by W. I. C., London, that two of the five strains, *viz.* A/India/29/54 and A/India/30/54 were found to be in the Q phase and the former was antigenically unique in that it differed from all other similar strains in their possession including the Nicholas strain isolated by Menon in Coonoor. These strains are being studied further as regards their mouse-adaptability with the ultimate object of utilising some of them for experimental vaccine production at this Institute.

(b) *Serological Diagnosis of Influenza*.—Haemagglutination-inhibition tests were carried out on six paired sera from suspected cases of influenza and only in three a four-fold or greater rise in antibody level could be demonstrated between the acute and convalescent phase samples of blood. The results are summarised in the following table:—

TABLE.

Patient's Name and Sera.	Anti ens.			
	PR8	Lee	FM1	Mainker.
Ramchandra	64/64	16/16	32/32	8/64*
Jayaram Babu	64/64	32/32	16/64*	
Dighe	16/16	16/16	32/64	8/32*

*Note.—The numerator shows inhibition titre of acute phase sample and the denominator shows that of the convalescent phase sample.

It will be seen from the above that use of standard antigens alone in the test set-up does not give the correct picture; local and recently isolated strains should be included whenever available. If the strain A/India/31/54 (Mainker) had not been included, the results of the test would have been inconclusive at least in two cases out of three.

(c) *Testing of Diagnostic Influenza Reagents on behalf of W.H.O.*—In the beginning of the year, Dr. A. M. M. Payne of the W.H.O. requested for a co-operative study in testing the above reagents prepared by Messrs. Phillips—Roxane of Amsterdam on behalf of the World Health Organisation. The reagents were supplied in a lyophilised form and comprised of allantoic fluid antigens, rabbit anti-sera and cholera filtrate. Initially all the antigens proved to be unsatisfactory as regards their haemagglutinating activity and were reported as such. At the end of the year, a second batch of similar reagents was received through Dr. A. M. M. Payne and they were tested not only for their sensitivity but also for their specificity. It was gratifying to note that they all proved satisfactory and all our newly isolated local strains could be correctly identified and typed by these reagents. There is no doubt that the use of such Standard Diagnostic Influenza Reagents will introduce uniformity amongst all Influenza Centres operating under W.H.O. throughout the world.

(d) The standard strains and previously isolated eight strains of influenza virus were maintained in regular egg-passages for utilisation either as antigens or for immunisation of roosters, in the preparation of diagnostic anti-sera.

XXIII. Further Studies on Laboratory Diagnosis of Small-pox.

(SETHI AND SOMAN).

(a) The technique of chorioallantoic inoculation was established during the last year which enabled us to differentiate cases of small-pox from chicken-pox. This year, the efforts were mainly concentrated in developing a suitable complement fixation technique in the diagnosis of early cases of small-pox, using a potent anti-vaccinal rabbit serum against an unknown antigen such as vesicle fluid or blood. In the preparation of the antivaccinal rabbit serum, two different kinds of vaccinal antigens were used for immunization of rabbits, (i) Testicular antigen and (ii) C. A. membrane antigen which worked very well. The serum reached a C. F. titre of 1 : 256.

Total number of cases investigated during the year was 161, out of which 121 were small-pox and 34 were chicken-pox. Microscopic Examination of smears from lesions for elementary bodies gave presumptive diagnosis in an hour in 28 cases out of 31 examined. 3 cases were missed due to lesion being in postular stage and the smear having shown lot of deposit after staining. In 2 cases with atypical rash, the elementary bodies were totally absent. In addition, vesicle fluids were mainly tested by complement fixation and chorio-allantoic inoculation tests were carried out wherever possible. Following table shows the number of cases investigated by either methods and the diagnosis arrived at, which was subsequently confirmed by clinical follow-up.

Methods of testing.	Results obtained in.	No. tested.	Diagnosis	
			Small-pox.	Chicken-pox.
Chorio-allantoic inoculation	... 48-72 hrs.	54	38	16
Complement fixation	... 24 hrs.	73	56	17

Laboratory diagnostic aid as outlined above was given to Port Health and Infectious Fever Hospital authorities in 7 cases to arrive at a correct diagnosis.

(b) *Significance of Viraemia and Viral Antigen in Blood of Small-pox cases.*— Presence of virus and viral antigen in blood in cases of small-pox beyond first four days was recently reported to be indicative of grave prognosis. Cases that generally recovered showed only a transient viraemia which disappeared after the development of secondary rash. In order to verify this statement it was necessary to investigate a few cases and with this in view arrangements were made with the Superintendent, Arthur Road Hospital, for the supply of blood of small-pox cases at very early stage of the disease, for inoculation on to the chorio-allantoic membrane to demonstrate viraemia and for performance of complement-fixation test for demonstration of viral antigen ; During small-pox season, 27 cases were investigated in this way out of which 18 had recovered and 9 proved fatal.

In all those cases that recovered, complement-fixation test was consistently negative, while egg culture test was negative in 14 cases out of 18. Out of 18 cases that recovered, eleven were vaccinated. 7 out of 9 fatal cases were of the primary haemorrhagic type—one was severe confluent type, and one developed secondary haemorrhages on 5th day. Among the fatal cases, only 3 were vaccinated. Egg culture test in all fatal cases showed presence of pocks which numbered from 2 to 50 but the complement fixation test was positive in only 5 out of 9 cases.

This appears to substantiate the observation that in cases of small-pox, presence of viraemia and the viral antigen beyond first four days of illness forebodes grave prognosis.

XXIV. Further Observations on the Biological Test for the Diagnosis of Rabies.

(SOMAN AND KHEMANI).

The biological test using white mice for the diagnosis of rabies was further extended to the year 1954 and was mainly carried out with negri-negative specimens. 63 brains were received for diagnosis of rabies out of which 44 were sent in glycerine and the rest in formol-saline. The results of 28 biological tests are summarised in the following table:—

<i>Total Number of Biological Tests.</i>	<i>Smear — Mouse —</i>	<i>Smear + Mouse +</i>	<i>Smear — Mouse +</i>
28	17	3	8

These results have again confirmed the previous observations that correct diagnosis of rabies on 8 specimens would have been missed if biological tests were not carried out. While the test is of great practical diagnostic value, it suffers from this drawback that the results only become available at the end of 8-12 days. The necessity for a quicker and more accurate method of diagnosis is obvious.

XXV. Studies on Complement Fixation Test in the Diagnosis of Rabies.

(SOMAN AND KHEMANI).

The present day methods of diagnosing rabies in animals consist of either demonstrating Negri bodies in brain tissue microscopically or by showing the presence of rabies virus in the brain by inoculation of mice. When Negri bodies are not demonstrable and mice are inoculated, it takes 10-15 days to observe the result, thus a person bitten is obliged to wait in suspense and anxiety for such a long period.

Attempts are made to develop a suitable complement fixation technique, where the unknown antigen in the form of infected brain could be titrated against a known positive and potent antirabies serum and the diagnosis arrived at in 24 hours. With this object in view, hyperimmune antiserum was prepared by first immunizing guinea-pigs with *different fixed virus strains*, to start with, and later using different street virus strains. Four tests were carried out by this method above described, out of which two gave correct results, in other two, the antigen was found to be anti-complementary.

If this method is properly standardized, the diagnosis of rabies would be made in 24 hours and it could be compared with the microscopic Negri-body test on the one hand and the mouse inoculation test on the other as regards its sensitivity and specificity. Further work is in progress.

XXVI. Potency Test on Hyperimmune Rabies Serum.

(SOMAN AND KHEMANI).

The antibody response to the first batch of six horses to immunization with Rabies Vaccine was observed by carrying out virus-neutralisation tests at certain intervals during the period of immunization, which extended to over six months. After the completion of the immunization schedule, only four horses out of six were bled and their sera pooled, the other two having shown irregular and insufficient response. This pooled antiserum when diluted between 250 and 600 was able to neutralise 100 M.L.D. of the C.V.S. fixed virus and protect 50 per cent. of the mice. On further concentration and purification and when tested by a similar procedure, it was able to neutralise the virus and protect 50 per cent. of mice in a dilution of 1 : 1200. Neutralisation test in mice was also carried out using serial ten-fold dilutions of the virus, which showed that the undiluted serum could protect against over 100,000 M.L.D. of the virus. Virus neutralisation tests were carried out as per standard procedures laid down by the W. H. O. Expert Committee on Rabies in their Monogram No. 23, using positive control serum of a known protective value. The final product was also sent to Prof. Lepine of the Pasteur Institute, Paris, who confirmed our results. Field studies are being carried out at six antirabic centres in Bombay State to test its protective value in severe cases of exposure due to rabid animals and the results are awaited.

XXVII. Studies on Street Virus of Rabies.

(SOMAN AND KHEMANI).

(a) Further comparative studies of the presence of virus in brain and salivary glands were carried out in 28 animals and the results for the year 1953 and 1954 are summarised in the following table :—

Year.			Total.	Brain+ Gland+	Brain+ Gland—	Brain— Gland—	Brain— Gland+
1953	...	...	29	8	8	13	0
1954		...	28	16	4	8	0
Total ...			57	24	12	21	0

The table shows that in 12 out of 36 positive cases the virus was not present in salivary glands. These results again bring out the fact that the presence of virus in the brain need not necessarily be accompanied by virus in the salivary glands at the time of death of the animal. This has an important bearing on transmission of Rabies in nature.

(b) In the previous year's report the salivary glands were titrated for virus content after varying periods of storage and it was thought that perhaps prolonged storage was responsible for the low titres obtained. During this year, the period of storage did not usually extend beyond a week or ten days and still virus content was found to be less than 10^{-5} . Only two dilutions: 10^{-1} and 10^{-5} of the salivary glands suspensions were tested. Sixteen salivary glands thus tested during this year showed a titre which was less than 10^{-5} . In order to develop and maintain a suitable challenge street virus strain having titre of $10^{-5.5}$ for potency tests on Avianized Vaccines, it appears it would be necessary to passage a few such strains in dogs by carrying out intramasseter inoculation.

XXVIII. Studies on Avianized Rabies Vaccine (Flurry Strain).

(SOMAN AND KHEMANI).

Three more batches of Flurry Vaccine were lyophilised and stored in the refrigerator. The results of infectivity titre in mice and the protective value in guinea-pigs of batch No. 11 were already reported in the last year's annual report. Similar tests were carried out on Batch No. 12 and its protective value compared with 20 per cent. sheep brain vaccine. The results obtained were irregular and inconsistent due to defective lyophilization. On retesting no live virus could be demonstrated in all three batches. Further work would be necessary to ascertain the effect of lyophilization at regular intervals on the live virus vaccine.

XXIX. Experimental Cholera in Rabbits.

(DUTTA AND HABBU).

A method has been described for inducing cholera in infant rabbits which closely resembles the human disorder. The disease is produced in ten-day old rabbits by infecting them intrainestinally with strain of Inaba or Ogawa. It is characterised by sévere diarrhoea, dehydration and occasional cramps in the muscles. The post-mortem examination shows congestion of the small intestines, desquamation of the epithelium at places and distention of the large intestine with watery fluid containing flakes of mucous membrane.

A method for evaluation of drugs against cholera is described. Chloromycetin, Aureomycin and Terramycin possess excellent preventive action, but they lack curative property even when administered at the early stage of the disease. The protective value of sulphaguanidine is less than the antibiotics, but is definitely superior to formocibazol.

XXX. Investigation on the Sedative and Hypotensive Principles in *Rauwolfia Serpentina*, Benth.

(SEN GUPTA, DALVI, TAMHANE AND DUTTA).

The roots of *Rauwolfia serpentina*, Benth, have been recognised for a long time in the indigenous system of medicine as a curative for many common ills and notably for insanity. Recently the drug has attracted world-wide attention due to its hypotensive and sedative activity.

The first scientific investigation on the plant was initiated by Siddiqui and Siddiqui, in the early thirties, who isolated five crystalline alkaloids. A number of other alkaloids have been isolated from the same plant by various workers during the last twenty years bringing the total number so far to twenty.

Ghosh had, however, reported in 1947 that the sedative principle of *Rauwolfia serpentina*, Benth was present in the 'Oleoresin' fraction which was free from alkaloids. The object of the present investigation was to isolate and characterise the sedative principle from *Rauwolfia serpentina* roots. It was noticed in the initial stages of the work that the 'oleoresin' fraction of Ghosh was not entirely free of alkaloids and that the sedative principle present in it was alkaloidal in nature. Working with large quantities of the oleoresin fraction it was found possible to isolate the active principle almost quantitatively. This was found to be identical with Reserpine which has been reported very recently to be the main sedative principle in *Rauwolfia serpentina*.

A number of pharmacological experiments carried out with this alkaloid showed that it possesses strong sedative and hypotensive activity.

An economical and simple method for the manufacture of this alkaloid has also been worked out.

XXXI. Detection and Determination of Saccharin in Tea.

(SEN GUPTA, MADIWALE AND BHATT).

Detection and determination of saccharin in samples of prepared tea, referred to this laboratory have been a problem for some time. Such samples generally four to six months old, when analysed by the available method for detection in non-alcoholic beverages invariably gave a positive indication for the presence of saccharin. On closer examination it was observed that decoctions of tea prepared in our laboratory, to which no saccharin had been added, gave a false positive indication when analysed by the above method.

In view of this, a recommended qualitative reaction for the detection of saccharin was studied and developed into a reliable and accurate method for detection and determination in such beverages as well as in medicinal preparations.

The method consists in precipitation of tannins by lead acetate, removal of caffeine and extraction of saccharin in a relatively pure state. Saccharin is next converted to phenolsulphonephthalein by heating with phenol sulphuric acid reagent at 135°-140° for two hours. On dissolving the melt in water and rendering the solution alkaline with caustic soda, a red-violet colour is produced indicating the presence of saccharin. For quantitative estimation, the intensity of the colour is measured spectrophotometrically at 560 mμ. The method is found to be accurate over the concentration range of 40 to 100 μg.

This method has never been found to give a false indication when applied to numerous samples of prepared tea which were examined immediately after preparation or after storage for 3 to 4 months.

XXXII. Antigenicity Tests for Protein Hydrolysate.

(DUTTA AND THADANI).

Protein hydrolysates are now being used widely in cases of patients who are unable to assimilate protein when given orally. Repeated and intermittent intravenous medication of hydrolysates of protein has raised a problem of anaphylaxis due to the presence of antigenic substances. A test has been devised for determining the antigenicity of protein hydrolysate used for intravenous administration. Groups of guinea-pigs weighing between 300 to 400 g. were sensitized to 2, 4, 6 and 9 hours acid digest of casein, the injections being given intraperitoneally every alternate days during a period of two weeks. After a lapse of 31 days since the time of last injection, the groups of animals sensitized to 2, 4, 6 and 9 hours digest of casein were challenged with 2 to 10 ml. (150 to 160 mg. of total solids) of 2, 4, 6 and 9 hours digest respectively. Another set of experiment was carried out in which animals sensitized to 2, 4, 6 and 9 hours digest were similarly challenged with 9 hour digest of casein. It was observed that guinea-pig sensitized with 2, 4 and 6 hours digest of casein when challenged with the sensitizing material showed reaction, but when challenged with 9 hours digest, no reactions were observed. It was also noticed when the animals sensitized to 9 hours digest were challenged with 9 hour digest of casein, showed reaction when higher doses were administered. On the basis of this experiment a method has been suggested for detecting increased susceptibility of casein hydrolysate to guinea-pigs.

XXXIII. Study on the Stability of Vitamins in Oral Preparations

EFFECT OF CRUDE LIVER EXTRACT, ALKALOIDS AND ENZYMES ON VITAMIN B₁.

(MEHTA, ANANTHANARAYANAN AND DUTTA).

Stability of aqueous alcoholic solutions of Thiamine hydrochloride in the presence of increasing doses of crude liver extract or Vitamin A, or Pepsin and Diastase, or the alkaloids Quinine Strychnine, or Choline and Methionine has been investigated. Certain aspects of the preservation problem of this vitamin when stored in the cold (0 to 5°) or in an atmosphere of nitrogen have also been carried out.

At room temperature the destruction of the vitamin was rapid in solutions containing crude liver extract or enzymes Pepsin and Diastase. Other substances tested did not alter the progressive deterioration of Vitamin B₁ to any considerable extent. The effect of crude liver extract was found to be quite appreciable on samples kept in the cold or in an atmosphere of nitrogen.

The results of these findings indicate the incompatibility of vitamin B₁ and crude liver extract or the enzymes, Pepsin and Diastase.

XXXIV. Indigenous Drugs in Experimental Diabetes.

(ANANTHANARAYANAN AND COLAH).

In the indigenous system of medicine a large number of drugs are recommended for the treatment of diabetes in human patients. The therapeutic effect of some of those drugs have been investigated in

experimental diabetes in animals. Alloxan which produces a selective necrosis of the β cells in the islets of langerhans was used for the production of diabetes in animals. Adult healthy rabbits were used in these experiments. Development of diabetes mellitus in these animals, was accomplished by injecting intravenously freshly prepared 5 per cent. solution of alloxan (300 mg/kg) in three divided doses at an interval of 15 minutes.

Indigenous drugs tested were (1) flowers of *Cassia auriculata*, Linn. (2) Seeds of *Eugenia Jambolana* and (3) leaves of *Vinca Rosea*. These drugs were finally powdered, and suspended in gum acacia solution (5%) and orally administered to the experimental animals, which were fasted previously. The drugs were administered at a dose level of 1 to 3 g. per day in two divided doses. Blood sugar estimations were carried out before and after the administration of the drugs; 4 to 5 animals were used for each drug. No hypoglycemic effect was observed with the drugs tested.

XXXV. Studies in the Synthesis of Vitamin B₁. Part I. Use of Acrylonitrile as an Intermediate for the Synthesis of the Pyrimidine Moiety of Thiamin.

(SHIRSAT AND MHASALKAR)

The present work was undertaken with a view to assess the feasibility of a procedure for the commercial manufacture of Vitamin B₁ described by the Russian workers Chelinstev and Benevolenskaya (1946), wherein cyanhydrin was used as one of the intermediates for the synthesis of the pyrimidine portion of the Thiamin molecule. The results of the Russian workers were substantially confirmed by us. Inspired by the encouraging results thus obtained, an attempt was made to investigate the possibility of synthesising the pyrimidine part of Thiamin by employing acrylonitrile instead of cyanhydrin. The results obtained by us in this direction established beyond doubt that the pyrimidine part can be thus synthesised at a much lesser cost and in better yield as compared with the Russian procedure. 2-Methyl-4-amino-5-ethoxymethylpyrimidine synthesised via acrylonitrile afforded the corresponding 5-bromo-methyl compound on treatment with hydrobromic acid. The bromo compound on condensation with 4-methyl-5-*beta*-hydroxyethylthiazole, prepared in this laboratory yielded the desired Thiamin-biomide hydrobromide, which on treatment with silver chloride afforded Thiamin-chloride hydrochloride in good yield, identical in every respect with an authentic sample of Thiamin-chloride hydrochloride.

XXXVI. Studies in the Synthesis of Vitamin B₁. Part II. Use of Lithium-Aluminium Hydride in the Synthesis of Pyrimidine Part of Thiamin.

(SHIRSAT AND MHASALKAR)

Hoping that the recently introduced unique and potent reducing agent, lithium-aluminium hydride might soon be available in commerce at a cheap and competitive price, it was felt worthwhile to investigate its utilization in the synthesis of the pyrimidine and thiazole parts of the Thiamin molecule. This was undertaken with a view to ascertain whether the manufacture of Thiamin could render a more simple and feasible operation as compared to the techniques hitherto followed. Pyrimidine portion was thus successfully

synthesised by the lithium-aluminium hydride reduction of the alkyl ester of 2-methyl-4-aminopyrimidine-5-carboxylic acid which afforded 2-methyl-4-amino-5-hydroxy-methylpyrimidine in good yield. This compound on treatment with hydrobromic acid afforded the corresponding 5-bromomethyl compound which on condensation with 4-methyl-5-*beta*-hydroxy ethylthiazole afforded Thiamin-bromide hydrobromide, identical in every respect with Thiamin-bromide hydrobromide obtained by employing 2-methyl-4-amino-5-bromomethylpyrimidine obtained by resorting to well established procedures.

XXXVII. Studies in the Synthesis of Vitamin B₁. Part III. Use of Lithium-Aluminium Hydride in the synthesis of Thiazole Moiety of Thiamin.

(SHIRSAT AND MHASALKAR)

Synthesis of the thiazole part of the Vitamin molecule by the condensation of thioformamide with *beta*-chlorolevulinic ester followed by lithium-aluminium hydride reduction of the 4-methylthiazole-5-acetic acid ester has been described in literature. However, with a view to investigate the feasibility of eliminating the use of thioformamide or of phosphorus pentasulphide required to be used therein, we synthesised 2-amino-4-methyl-5-*beta*-hydroxy ethylthiazole and 2-mercapto-4-methyl-5-*beta*-hydroxyethylthiazole by the above described procedure, wherein instead of employing thioformamide, the condensation was effected with thiourea and with ammonium dithiocarbamate respectively. The amino group or the mercapto group in 2 position of the thiazole ring was successfully replaced by hydrogen by resorting to certain modifications of the classical procedures, to obtain 4-methyl-5-hydroxyethylthiazole in good yields in both the cases, the compounds being identical in every respect with 4-methyl-5-hydroxyethylthiazole obtained by well established procedures.

XXXVIII. Chemotherapy of Bacterial Infections : Synthesis of N¹ Benzimidazolyl Sulphanilamides and Metanilamides.

(SHIRSAT AND BAPAT).

During the course of synthesising sulphonamide derivatives of benzimidazoles (Annual Report for the year 1953) it was found necessary to study the basic character of the benzimidazole nucleus in order to determine the chemical structure of the sulphonamide derivatives obtained therefrom. It was found that 5-amino-2-alkylbenzimidazoles are more basic than pyridine as well as ammonium hydroxide. On the other hand 5-amino-2-arylbenzimidazoles were found to be more basic than pyridine but less basic than ammonium hydroxide.

As a result of these studies, it has been proved that the aryl sulphonyl chloride such as acetsulphanilyl chloride or *m*-nitrobenzensulphonyl chloride condense with the 5-amino-benzimidazole and 5-amino-2-alkylbenzimidazole at 5 position in preference to the secondary nitrogen of the imidazole ring, when the reaction is carried out in pyridine medium. Attempt at condensation with 2 molecular equivalents of the aryl sulphonyl chloride per molecule of the 5-aminobenzimidazole compounds also failed to induce the aryl sulphonyl chloride to condense with the secondary ring Nitrogen of the imidazole ring. However, condensation of aryl sulphonyl chloride with 5-aminobenzimidazoles in aqueous medium in the presence of sodium

bicarbonate and a little acetone yielded very interesting results. 5-amino-2-alkyl-benzimidazoles on treatment with 2 moles of acetsulphanilyl chloride in aqueous medium in the presence of sodium bicarbonate furnished the desired diacetsulphanilyl derivatives wherein one of the acetsulphanilyl groups was linked up with the 5-amino group, the other having condensed with the secondary nitrogen of the imidazole ring. Condensation with 1 mole of acetsulphanilyl chloride however, furnished the monocondensation product wherein the acetsulphanilyl group had condensed with the 5-amino group in preference to the secondary nitrogen of the imidazole ring. This behaviour of 5-amino-benzimidazole molecule has been shown to be parallel to the behaviour of 2-aminothiazole. The mono and dicondensation products obtained above, yielded on hydrolysis the corresponding 5-arylsulphonamide compounds in both the cases. 5-Amino-2-Arylbzenzimidazoles however, failed to furnish the discondensation products, but yielded the mono-condensation products irrespective of the number of mol. equivalents of acetsulphanilyl chloride employed.

Simple 2-alkylbenzimidazoles on treatment with acetsulphanilyl chloride in pyridine or aqueous medium afforded the 1-sulphanilyl derivatives which on attempted hydrolysis yielded the starting compounds, 2-alkylbenzimidazoles. 2-Arylbzenzimidazoles failed to condense with acetsulphanilyl chloride under the various experimental conditions tried.

The acetsulphanilyl group attached to the secondary nitrogen of the imidazole ring could be easily hydrolysed by heating with alkali, but was quite stable to the action of pyridine.

m-Nitrobenzenesulphonyl chloride was condensed with various 2-alkyl-5-aminobenzimidazoles; 2-aryl-5-aminobenzimidazoles and with 2-pyridyl-5-amino-benzimidazoles to obtain the corresponding 2-substituted-5-N-(*m*-nitrobenzenesulphonyl)-amidobenzimidazoles. No dicondensation products could be isolated in this case under the experimental conditions tried. The various nitrobenzene-sulphonyl derivatives on reduction with tin and hydrochloric acid furnished the corresponding amino compounds in good yields.

XXXIX. Study of Serum Proteins Fractions in Cirrhosis of Liver.

(VENKATARAMAN)

Serum proteins and their changes in disease have been extensively studied by salt fractionation methods and more recently by electrophoresis. Several workers have shown by comparative studies that the so-called albumin fraction obtained by fractionating serum with 22-23 per cent. sodium sulphate contains an appreciable portion of the alpha globulin. It has also been shown that a true picture of albumin and the various globulins can be obtained by chemical fractionation techniques which have been adjusted to electrophoretic standards. Thus, at a concentration of 15-16 per cent. sodium sulphate only the gamma globulin is precipitated; by increasing the concentration of sodium sulphate slightly to 19-20 per cent. both gamma and beta globulins come down, while at a concentration of 26-27 per cent. all the globulins are precipitated leaving only the albumin portion in solution. This simple technique of fractionation which has been found to give results reasonably consistent with those obtained by electrophoretic analysis will be very useful in the study of serum protein fractions in disease.

A number of serum samples from cases of cirrhosis of liver have been studied with reference to their true albumin, alpha, beta and gamma globulins by salt fractionation. Pooled serum samples from normal donors from the Blood Bank have been used as a basis for comparison.

The cases so far investigated conform to a typical picture with considerable decrease in albumin and increase in globulin concentrations. The individual globulin fractions (α , β , γ) are also found to be significantly altered. While the alpha component shows a definite decrease, the gamma globulin is very much elevated. The results obtained compare well with published data using electrophoretic analysis.

XL. Preparation of Highly Potent Anti-human Rabbit Serum.

(MAHAL AND KULKARNI)

The utility of Anti-human serum was formerly restricted to the medico-legal use only, but with the discovery of the Coomb's Test the utility of this serum is extended to tests for cases of isosensitization, acquired hæmolytic anæmia etc. So the production of a reliably high potent serum is a necessity of every blood bank. Various methods described in the literature were tried and satisfactory serum could only be obtained by the method described by Proom (Jou. Path. Bact. (1943), 55, 419). With modification in the final adjustment of pH of alum precipitated serum to 4.7 instead of 6.5 as in original method, a more potent antiserum was obtained. The final titre was as high as 1 : 32000 as against 1 : 16000 mentioned by Proom. This titre was further enhanced by the method suggested as by Woolton [Nature (1950), 165, 730] and final precipitin titre of 1 : 45000 was obtained. This serum could be used both for medico-legal work and also as a Coomb's Reagent after preliminary absorption and titration.

XLI. Haemopoetic Response following Blood Donation in Professional Blood Donors.

(KARKHANIS AND KULKARNI).

The regeneration of blood following blood donations was previously studied in those who donated blood once in a way. Presently majority of the blood donors are professional ones and donate blood very frequently and nutritive value of their diet is also low. So with a view to assess the blood regeneration in these persons, blood studies were carried out on 48 professional blood donors. Haemoglobin level, packed cell volume and Red blood cell count were determined before and at regular intervals after one blood donation and also after repeated blood donations. It was found that the blood regeneration was slower in these persons than those who give blood once in a way. The maximum drop in Haemoglobin level and R.B.C. Count is observed after 2 days of blood donation. The hæmoglobin regeneration takes about 17 days and R.B.C. regeneration to original level about 7 days. Administration of Iron compounds by mouth enhances the regeneration rate considerably. The regeneration gradually becomes delayed as the frequency of donation or the number of donation increases.

XLII. Amino Acid Composition of Human Milk.

(RAJ, WAGLE AND RADHAKRISHNA RAO)

The qualitative examination by paper chromatography of amino acids in about 35 samples of human colostrum and transitional milk collected 2 to 10 days post-partum, was reported last year. Further work on the essential amino acids analyses of human milk was carried out by microbiological assay technique.

Seventeen samples of milk of mothers belonging to low economic groups have been analysed. Of these, three samples were from anaemic mothers and two from mothers whose children suffered from liver enlargement. The protein content of majority of the samples is in agreement with the minimum values (0.8 per cent. protein) reported in the literature. Some of the samples from apparently normal mothers, however, contained proteins as low as 0.54 per cent. While the values for fat, carbohydrates, ash and moisture compare favourably with those observed by others, the values for calcium, phosphorus and iron are slightly lower in most of the cases.

All the samples of milk were assayed for the essential amino acids and also for cystine. The values of most of the amino acids in large number of samples are similar to those found by others. Comparison has also been made on values calculated for 100 ml. of milk, as this is of more practical significance from the nutrition view-point. For convenience, the samples have been classified arbitrarily into four groups *viz.* 1 to 3 months, 4 to 6 months, 7 to 12 months and the rest, depending upon the lactation period. There were 3, 8, 3 and 3 samples in each group respectively. The samples of milk collected during the early months of lactation (1-3 months) showed comparatively higher content of most of the essential amino acids. No definite conclusions can be drawn from the study of such a small number of samples. It is therefore proposed to continue the analyses of large number of samples of milk from different economic groups.

Similar analyses on the samples of urine, particularly of cirrhotic children, would be interesting.

XLIII. Influence of Fermented Food-stuffs on Fatty Infiltration of the Liver in Rats.

(WAGLE, PATEL AND RADHAKRISHNA RAO).

As reported last year, two experiments were planned to study the effects of supplementation by fermented food, *viz.* 'Iddlee' at 50 to 100 per cent. replacement of dietary protein.

Experiment No. 1.—50 per cent. replacement of protein by 'Iddlee'.

Thirty weanling rats (all male) of the Haffkine Institute in-bred strain weighing about 30-35 g., were distributed in 5 groups of six animals each. The control group received a high fat low protein diet (HFLP—30 per cent. fat and 10 per cent. casein). Of the four groups, one group was given 'Iddlee' and the second group an unfermented preparation at 50 per cent. replacement of protein in HFLP diet from the start of the experiment. These two groups were designed to study the 'preventive' effect of 'Iddlee' on the production of fatty infiltration in the livers. The remaining two groups were kept for 40 days on HFLP diet and then transferred on 'Iddlee' and unfermented preparation. The latter formed the 'curative' groups.

The composition of 'Iddlee' consisted of 4 parts of black gram powder and one part of rice. This mixture was allowed to ferment for 15-20 hours at ordinary room temperature. The rats were sacrificed at the regular intervals. The results are set in Table I.

Experiment No. 2.—100 per cent. replacement of protein by 'Iddlee' (no casein present in the diet).

Thirty-six weanling rats (male) of the abovementioned strain were divided into two groups of 18 animals each. Only 'preventive' tests have been carried out at 100 per cent. replacement by 'Iddlee'. Other experimental details are similar to those in Experiment 1. The results are summarised in Table II.

Table I.

Group	No. of days on test diet.	Gain in weight g.	Plasma proteins g.	g. per 100 g. of the body weight.		
				liver wt.	liver fat.	liver protein.
Control	...	40	19.2	5.0	9.58	4.18
		60	41.0	5.0	10.64	4.3
		75	44.0	5.3	11.9	4.6
		90	46.7	5.2	11.0	4.3
Preventive	Unfermented preparation.	40	38.5	5.1	6.88	1.12
		60	26.5	5.8	8.04	1.47
		75	59.0	5.45	6.84	1.5
	'Iddlee'	40	46.0	5.25	6.4	0.68
		60	22.0	6.2	6.4	0.67
		75	98.0	6.1	6.1	0.77
	Unfermented preparation.	40+30	60.0	4.4	8.4	2.2
		40+45	37.0	4.6	7.35	1.62
		40+60	64.5	4.8	6.1	1.34
Curative	'Iddlee'	40+30	60.0	5.3	7.9	1.64
		40+45	35.0	5.2	6.1	1.60
		40+60	65.5	5.0	6.6	1.26
	Unfermented preparation.	40+30	60.0	5.3	7.9	1.64
		40+45	35.0	5.2	6.1	1.60
		40+60	65.5	5.0	6.6	1.26

The values in the above Table are average of two rats in each group.

Table II.

Group	No. of days on test diet	Gain in weight g.	Plasma proteins g.	g. per 100 g. of the body weight.		
				liver wt.	liver fat.	liver protein.
Unfermented preparation.	...	25	4.0	4.2	3.5	1.8
		60	24.0	4.35	7.8	2.0
		75	31.0	4.8	7.5	2.35
'Iddlee'	...	25	3.5	4.30	3.8	1.24
		60	31.0	4.80	5.6	1.60
		75	36.0	5.1	5.1	1.82

The values in the Table are average of 6 rats in each group.

The results in the Table I clearly indicate the beneficial effects of 'Iddlee' in preventing the deposition of fat in the livers. Similar effect is not so pronounced in the 'curative' groups. This suggests that the period for which the animals were kept on 'Iddlee' in the latter case, is probably not enough to clear off the already deposited fat. The control group on 10 per cent. casein showed very heavy fatty infiltration. Unfermented preparations were without much benefit. The plasma proteins and liver weights also showed an improvement in experimental as compared to the control groups.

Histological examination of liver sections of control animals showed as usual moderately severe fatty infiltration leading to fibrosis in few cases. Rats on unfermented preparation (both 'preventive' as well as 'curative') showed fatty infiltration but no fibrosis of the liver. In the case of 'Iddlee' group ('preventive') liver sections showed normal lobular pattern with only occasional fatty infiltration of the parenchymal cells here and there. Thus, both the biochemical and histological observations are in good agreement.

In view of the above observations (Table I), the second Experiment was planned to study only the 'preventive' effect of the 'Iddlee' at a 100 per cent. replacement of casein protein. Although the 100 per cent. replacement of protein by 'Iddlee' helped in preventing deposition of fat in livers, the effect was not so marked as at 50 per cent. replacement. Moreover, the gain in weight was also larger in Experiment No. 1. Animals of Experiment No. 2 showed fall in plasma and liver proteins as compared to Experiment No. 1. This suggests that the combination of proteins (50 per cent. casein plus 50 per cent. 'Iddlee' protein) is superior to either of the two alone.

The histological examination of the liver sections of the animals in this group is in progress.

Before any explanation could be offered, further work is considered necessary.

XLIV. Experimental Hepatic Necrosis.

(AMBEGAOKAR, PATEL, KRISHNAN AND RADHAKRISHNA RAO).

(a) *Effect of different levels of dietary yeast in the production of hepatic necrosis.*—It has been observed last year that in rats fed 'yeast necrogenic diet' with yeast levels varying from 20 to 60 per cent. only those receiving at 30, 25 and 20 per cent. levels showed massive hepatic necrosis. It was intended to confirm these results and see whether massive haemorrhagic necrosis can be produced regularly at lower levels of yeast feeding.

Two groups of rats each of ten, were fed 'yeast necrogenic diet' at 15 and 20 per cent. yeast levels respectively. The rest of the diet was similar to that used in our previous experiments.

Unlike last year, the animals were not killed at regular intervals, all of them died of massive hepatic necrosis within 30 to 40 days of feeding the test diets. These findings are in agreement with those reported by Himsworth and Glynn (1944). Nine out of ten animals maintained on diet containing yeast at 15 per cent. level, developed massive hepatic necrosis of grade 3 and one died of necrosis of grade 2. Amongst nine animals maintained on 20 per cent. yeast level, six animals died of massive hepatic necrosis of grade 3 and three died of necrosis of grade 2. Thus, massive hepatic necrosis was produced regularly in rats fed yeast at 20 and 15 per cent. levels.

(b) *Analysis of Yeast.*—Sulphur content of yeast is an important factor, beside others, in favouring the production of dietic hepatic necrosis. In view of this, the yeast used in the above diets was analysed with respect to its sulphur distribution gravimetrically by differential oxidation method of Lugg (1938) and Masters (1939). The total sulphur content was found to be 0.350 g. per cent., methionine sulphur, 0.108 g. per cent. and cystine sulphur plus sulphate sulphur, 0.242 g. per cent. The values for methionine and cystine are in close agreement with those obtained by us by colourimetric method of Sullivan and by microbiological assay and are similar to those given by Lindan (1951) for Baker's yeast—a strain of yeast reported to be most suitable for the production of hepatic necrosis.

(c) *Influence of choline chloride supplement on the incidence of hepatic necrosis.*—The role of choline in transmethylating reactions *in vivo*, particularly in the metabolism of sulphur amino acids, is well known. In order to study the influence of choline on the production of hepatic necrosis, the following experiment was carried out. The rats in this group received the diet containing yeast at 20 per cent. level and choline chloride supplement 5 mg. per rat per day, the rest of the diet remaining the same as in the previous experiment. The incidence of massive hepatic necrosis was diminished slightly in this group. Only four animals out of ten developed massive necrosis of grade 3, while six animals died of necrosis of grade 2.

List of Papers Published from the Haffkine Institute during the Year 1954.

1. BHAGAVAN, N. V., NIMBKAR, Y. S. & RAO, S. S.—Antigenic Analysis of *Pasteurella*. *Current Science* (In Press).
2. DESHPANDE, P. D. & RAO, M. V. R.—Nitrogen Complex and Amino Acid Composition of (i) Amaranth (*Amaranthus gangeticus*); (ii) Aconite Bean (*Phaseolus Aconitifolius*). *Ind. J. Med. Res.* 42, 77-83, 1954.
3. DESHPANDE, P. D. & RAO, M. V. R.—The Biological and Supplementary Nutritive Value of (i) Amaranth (*Amaranthus gangeticus*) and Aconite Bean (*Phaseolus aconitifolius*). *Ind. J. Med. Res.* 42, 515-520, 1954.
4. DOGRA, J. R. & MODI, B. G.—The Rapid Rat Test for Pregnancy. *J. Ind. Medical Profession.* 1, 65- , 1954.
5. DOGRA, J. R. & MODI, B. G.—Validity of Biologic Pregnancy Tests : An Analysis of 1660 Cases. *J. Ind. Medical Profession.* 1, 277-279, 1954.
6. DUTTA, N. K. & ANANTANARAYANAN, K. G.—Release of Histamine from Skeletal Muscle by Snake Venoms. *Brit. J. Pharm. Chemo.* 9, 408-412, 1954.
7. DUTTA, N. K. & TAMHANE, R. G.—Effect of Atropine Pethidine, Procaine, Quinidine and Prophen Pyridamine Maleate on the Histamine induced Gastric Acidity in Cats. *Ind. J. Med. Res.* 41, 45-50, 1953.
8. GOVINDAN, K. K., DESHPANDE, P. D. & RAO, M. V. R.—Studies on Lipotropic Factors. Part III. Production of Fatty Livers—Influence of Dietary Fat. *Ind. J. Med. Res.* (In Press).
9. GOVINDAN, K. K. & RAO, M. V. R.—Studies on Lipotropic Factors. Part IV. Influence of Diet on Liver Phospholipids.
10. GOVINDAN, K. K., DUTTA, N. K. & KAMATH, S. R.—The Supplementary Nutritive Values of Cane Sugar, Cane Gur and Palm Gur when Incorporated in Complete Diet. *Ind. J. Pharmacy*, 16, 69-70, 1954.
11. KULKARNI, M. E., RAO, SHANTA S. & RAO, S. S.—Use of Oudin's Gel Diffusion Technique for Determination of Purity of Proteins and Polysaccharides. *Current Science.* 23, 190-191, 1954.
12. KUNDAJI, TARA R. & RAO, M. V. R.—Studies on Blood and Tissue Proteins. A Modified Specific Gravity Method for the Estimation of Plasma Proteins and Haemoglobin. *Ind. J. Med. Sc.*, 8, 98-101, 1954.
13. KUNDAJI, TARA R. & RAO, M. V. R.—Supplementary Nutritive Value of Some Subsidiary Cereals. *Current Science*, 23, 93-94, 1954.
14. MADIWALE, M. S. & DUTTA, N. K.—Assay of Folic Acid. *Ind. J. Pharmacy.* 16, 18-21, 1954.
15. MEHTA, C. J.—Is Chlorophyll a Deodoriser, *Medical Digest*, 22, 422-426, 1954.

16. PATEL, S. M., KUMTA, U. S. & RAO, M. V. R.—Stability of Vitamin A in Aqueous Dispersions and in Oils. *J. Sc. Ind. Res.* (In Press).
17. RAO, S. S.—Review for Biochemical and Allied Research in India on the subject "General Microbiology including Antibiotics," for 1952. *Proc. Soc. Biol. Chemists of India*, 23, 1952.
18. RAO, S. S.—General Microbiology including Antibiotics. *Annual Review of Biochemical and Allied Research in India for the year 1953.* *Proc. Soc. Biol. Chemists of India*, Vol. 24, 1953.
19. RAO, SHANTA S. & LAHIRI, D. C.—Studies on Changes Associated with Conversion of Diphtheria Toxin into Diphtheria Toxoid with Formalin and Heat. *Ind. J. Med. Res.* 41, 33-43, 1953.
20. RAO, S. S., KULKARNI, M. E., COOPER, S. N. & RADHAKRISHNAN, M. R.—Analysis of Proteins of Bovine Lens Vitreous and Aqueous by Electrophoresis and by Oudin's Gel Diffusion Technique. *Brit. J. Ophthalmology.* (In Press).
21. SENGUPTA, M. C., MADIWALE, M. S., BHATT, J. G.—Estimation of Saccharin in Prepared Tea. *Ind. J. Pharm.* (In Press).
22. SESHADRI, S. PATEL, S. M. & RAO, M. V. R.—Toxin Principles of 'Khesari' (*Lathyrus sativus*). *Current Science* (In Press).
23. SETHI, K. & SOMAN, D. W.—Laboratory Diagnosis of Small-pox. *Ind. Med. Gazette* (In Press).
24. SIDDONS, L. B.—Screening of Antimalarial Compounds against *Plasmodium Berghe*. *Ind. J. Malariology*, 7, 41-52, 1953.
25. SOKHEY, S. S., WAGLE, P. M. & HABBU, M. K.—Treatment of Bubonic Plague with Sulphonamides and Antibiotics. *Bull. W. H. O.*, 9, 637-643, 1954.
26. SOMAN, D. W.—A Report on the Isolation of Two Strains of Influenza B. Virus in India, *Ind. Med. Gazette* (In Press).
27. SOMAN, D. W.—A Review of the Present Position of Tsutsugamushi Disease (Scrub typhus) in Bombay City and Suburbs. *J. Ind. Med. Assn.* 23, 389-394, 1954.
28. SOMAN, D. W.—Q Fever in India: Serological Evidence. *Ind. J. Med. Sc.*, 8, 698-703, 1954.
29. VENKATARAMAN, A.—The Estimation of Sodium in Biological Fluids. (A Modified Uranyl Reagent and Simple Procedure for the Determination of Sodium in 0.1 ml. of Serum or other Body fluids are Described). *J. Sc. Ind. Res.* 13-B, 713-718, 1954.
30. WAGLE, P. M. & SEAL, S. C.—Application of DDT, BHC and Cyanogas in the Control of Plague in India, *Bull. W. H. O.*, 9, 597-614, 1953.

P. M. WAGLE,

Parel, Bombay, April 13, 1955.

Director, Haffkine Institute.

