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REPORT
OF
THE HAFFKINE INSTITUTE

For the Year 1953

BY

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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.

REPORT OF THE HAFKINE INSTITUTE FOR THE YEAR 1953.

CONTENTS.

PART I.						PAGE.
GENERAL.						
I.	Staff	..	..	..	..	1
II.	General Review	..	..	..	..	2
III.	Department of Vaccines	..	..	..	..	5
	A. Plague Vaccine	..	..	..	..	6
	B. Cholera Vaccine	..	..	..	..	8
	C. T. A. B. Vaccine	..	..	..	..	8
	D. Media Section	..	..	..	..	9
	E. Section of Public Health, Chemistry and Bacteriology	..	..	..	..	10
IV.	Department of Antitoxins and Sera	..	..	..	..	10
V.	Department of Entomology	...	..	..	..	14
	A. Snakes	..	..	..	..	14
	B. Examination of Bombay City Rats	..	..	..	..	15
	C. Examination of rats and fleas from Steamers and Other sources	..	..	..	..	17
VI.	Department of Clinical Pathology and Diagnostic Reagents	..	..	..	..	24
	1. General Bacteriological Work	..	..	..	..	25
	2. Diagnostic Reagents	..	..	..	..	27
	3. Histopathology Section	..	..	..	..	28
	4. Special Investigations	..	..	..	..	30
	5. Training and Research Scholars	..	..	..	..	31
	6. Research	..	..	..	..	31
VII.	Department of Pharmacology	..	..	..	..	32
VIII.	Department of Chemotherapy	..	..	..	..	37
IX.	Department of Biochemistry	..	..	..	..	39
X.	Department of Virus Diseases including Rabies	..	..	..	..	40
XI.	Blood Bank Department	..	..	..	..	45
XII.	Training of Health Personnel	..	..	..	..	47
XIII.	Critics Club	..	..	..	..	48
XIV.	Miscellaneous	..	..	..	..	49
XV.	Income and Expenditure	..	..	..	..	50

PART II

RESEARCH WORK

I.	Nutritional Requirements of P. Pestis	..	..	..	51
II.	Passive Immunity Tests with Anti-cholera Sera	..	..	..	52
III.	Bactericidal Titre of Anti-cholera Sera as a Measure of the Antigenic Value of Cholera Vaccine.	..	..	..	54

	PAGE.
IV. 44°C. Mackonkey Test for Identification of <i>B. Coli</i> in Water Analysis.	54
V. Effect of Bentonite (Montmorillonite) on the Antibodies of Concentrated Immune Horse Serum Globulins.	55
VI. Coimon Antigens in the Venoms of Indian Poisonous Snakes viz. Cobra, Krait, Russell's Viper and <i>Echis Carinata</i> .	56
VII. Action of Crystalline Trypsin on Crystalline Egg Albumin ..	57
VIII. Fractionation of the Soluble Antigens of Plague	57
IX. Optimum Time Required for Action of Venom at Different Levels in Cobra Venom.	57
X. Survival Time as Affected by the Interval Between the Administration of Venom and Antivenine.	58
XI. Test Toxin and Standardization of Anti-tetanus Serum	59
XII. Pulcidal Value of Pyrethrum	60
XIII. Entomological Survey of the Swadeshi Mills, Kurla	60
XIV. Some Observations on Snake Blood	63
XV. Sex Ratio in Flea Collections	65
XVI. Chloromycetin in Typhoid Fever	65
XVII. Pregnancy Test	65
XVIII. In Vitro Activity of Indian Medicinal Plants	67
XIX. Anerobically Grown Typhoid Organisms in Widal Test	67
XX. Tropical Eosinophilia—An Experimental Study on Laboratory Animals.	67
XXI. Serodiagnosis of Syphilis—A Comparative Study	68
XXII. Use of Dyes in Serodiagnostic Tests for Syphilis	69
XXIII. Preservation of Rabies Virus by Freeze-Drying	70
XXIV. Immunity Response to Semple's Antirabic Vaccine in Man ..	70
XXV. Antibiotic Properties of Indigenous Plants	73
XXVI. Studies on Influenza Viruses	73
XXVII. Further Studies on the Laboratory Diagnosis of Small-pox ..	74
XXVIII. Isolation of Herpes Virus	75
XXIX. Studies on Street Virus of Rabies	75
XXX. Studies on Avianized Rabies Vaccine	76
XXXI. Further Observations on the Biological Test for the Diagnosis of Rabies.	77
XXXII. Further Observations on the Keeping Qualities of Yellow Fever Vaccine.	78
XXXIII. Post-Vaccinal Reactions	78

	PAGE.
XXXIV. Investigation on the Amino Acid Contents of Casein Hydrolysate for Intravenous Use.	78
XXXV. Assay and Stability of Folic Acid	79
XXXVI. A Study of the Pharmacological Properties of Chaksine—An Alkaloid Derived from the Seeds of Cassia Absus Linn.	79
XXXVII. A Study on the Stability of Vitamins in Oral Preparations—Effect of Vitamin A, Liver Extract, Alkaloids and Enzymes on Thiamine Hydrochloride.	79
XXXVIII. Placental Transmission of 4 : 41—Diamino Diphenyl Sulphone (DDS) in the Rabbits.	80
XXXIX. Chemistry of the Schiff Bases of Sulfonamides	80
XL. Chemistry of O-Hydroxy Sulphonamides	81
XLI. Antimalarial Activity of Sulphonamides	82
XLII. Chemotherapy of Bacterial Infections—Synthesis of N ¹ -Benzimida-zolyl Supphanilamides and Metanilamides.	82
XLIII. Effect of Different Levels of Dietary Yeast in the Production of Hepatic Necrosis	83
XLIV. Amino Acid Composition of Human Milk	84
XLV. Influence of Fermented Foodstuffs on Fatty Infiltration of the Livers.	84
List of Papers published from the Haffkine Institute during the year 1953.	85

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1953.

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

Dr. A. K. Hazra, M.B.B.S. (Bom.), D.T.M. (Cal.), B.M.S. (I), Assistant Director.
I/c Department of Antitoxins and Sera from 20th June 1953.

Dr. K. Ganpathi, B.A., D.Sc. (Madras), B.M.S.(I).
I/c Department of Chemotherapy up to 5th January 1953.

Lt. Col. J. R. Dogra, M.D., Ch. B. (Edin.), D.T.M. (Liverpool).
I/c Department of Clinical Pathology.

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

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

Shri M. V. Shirsat, M.Sc. (Bom.), Officiating Assistant Director.
I/c Department of Chemotherapy from 6th January 1953 to 31st September 1953.

Dr. C. V. Deliwala, M.Sc., Ph.D., Officiating Assistant Director.
I/c Department of Chemotherapy from 1st October 1953.

II. General Review.

The Institute functioned as usual as (i) a centre for the manufacture of plague, cholera, typhoid and antirabic vaccines; (ii) a centre for the manufacture of sulphathiazole, vitamin tablets, casein hydrolysate and other parenteral solutions for the use of Government Hospitals and Institutions in the State; (iii) an analytical laboratory for the enforcement of Drugs Act, 1940, and as (iv) the provincial Bacteriological Laboratory for the Bombay State. In spite of the heavy pressure of routine manufacturing activities, research work was carried out by most of the senior members of the staff on problems connected with the activities of the Institute. The Institute collaborated with the Department of Nutrition in investigating the cause of the outbreak of "Epidemic Dropsy" at Nadiad in Kaira District.

The outbreak of "Epidemic Dropsy" at Nadiad again stresses the need for creation of a mobile unit in this Institute for conducting field and other studies on urgent health problems of the people in close association of the public health department.

The demand for the plague vaccine continued to be on the downward grade, issues this year being even lower than those of the last year. Only about one million millilitres of vaccine was issued this year as compared to about five million millilitres of vaccine last year. But although the demand for plague vaccine was lower owing to the reduced incidence of the disease in the country, the demand made for cholera vaccine was very heavy and unprecedented in the history of the Haffkine Institute. This was due to the occurrence of severe outbreak of cholera infection all over the country during the last monsoon. The demands made in the Institute were, however, promptly met, thanks mainly to commendable efforts made and devotion to the duty shown by all members of the staff of the Vaccine and Allied Departments. 19.8 million millilitres of cholera vaccine was supplied during the year as against 3.8 million millilitres in 1952.

As a result of higher cholera incidence in the State this year, the demand for parenteral solutions was also higher, one lakh bottles having been issued this year as compared to sixty-eight thousand in last year. The demand for these solutions is on the increase year by year.

The work on the nutritional requirements of plague bacillus was continued. Further work has shown that the action of activated animal charcoal "nuchar" in enhancing the growth of *P. Pestis* in casein hydrolysate is not due to any changes brought about in the redox potential of the medium, but is due to enhancement of the reducing systems of bacteria during their growth and metabolic activities.

The use of antitoxins and sera has been steadily increasing. The demands are coming not only from this State, but also from other States of the country and also from foreign countries. The acquisition of the stud farm at Pimpri has enabled the Institute to increase the production of antitoxins by over fifty per cent. of the normal average production of the previous years. Further increase is necessary in view of the higher demands for antitoxins and sera, but will depend primarily on the development of full fledged laboratory at Pimpri with provision of necessary technical staff. The need for such development need hardly be stressed in view of the important role the antitoxins, sera and toxoids play in the maintenance of public health.

Investigation is being conducted in the Department of Antitoxins and Sera on the composition of venoms of different snakes. The work, if successful, may enable us to elucidate the mechanism of the action of these venoms in human beings and possibly produce an antivenene of higher potency. The work has not, so far, reached a stage where any conclusions can be drawn.

The Institute is manufacturing the new Diphtheria prophylactic 'N.A.F.T.' on a small scale. Confirmatory results from other laboratories as to its value are awaited before its regular manufacture is undertaken at the Institute.

The Department of Pharmacology met the increasing demand for testing of drugs from this as well as other States and carried out investigations on problems arising out of such examinations. In all 1,909 samples were examined during the year as against 1,524 in 1952 and 1,284 in 1951. This Department is now testing drugs under the Drugs Act, 1940, not only for this State but also for East Punjab, Delhi, Uttar Pradesh, Madhya Pradesh, Bihar, Saurashtra and Assam. Three other States have also requested the Government to let this Department serve as an analytical laboratory for their States. This request has now been accepted by Government.

Twenty-seven per cent. of the samples sent for quality control this year were not found up to standard as against 33 per cent. in the previous year. 1,213 samples were examined under the Drugs Act and of these 35 per cent. were found to be of non-standard quality, as compared to 37 per cent. in 1952. There was thus no appreciable improvement noticed in the quality of drugs exposed for sale. It is hoped that with co-operation of medical practitioners, and people in pharmacy trade and industry, and with more stringent drug control measures, it would be possible to improve the quality of drugs in this country.

An idea about the hoax played on the public by selling spurious drugs may be obtained by citing a few examples detected during the examinations. In a few cases sulphanilamide powder was sold as Procaine Penicillin; starch chalk or kaolin as testless quinine; sodium bicarbonate as Chloromycetin; and a yellow inert powder as Aureomycin. The imitation of the genuine sample was so perfect that it would have been impossible for any but a trained eye to detect the fraud. Amongst the tinctures examined for the Prohibition Department, some were found to be nothing but coloured alcohol. Necessity for stringent control needs no emphasis.

A study on the stability of Vitamin B¹ has shown that the potency of this vitamin is almost completely lost at room temperature in course of a year when combined with crude (oral) liver extracts and vitamin A; Folic Acid was also found to be unstable in complex multivitamin preparations. An investigation into the manufacture of protein-hydrolysate has enabled the Department to prepare a product which is expected to be superior to the one that is at present being manufactured in the Institute.

The Department of Entomology has continued the study to evaluate the pulicidal power of pyrethrum formulations. Different formulations have been tested and some of the formulations have given results comparable to DDT. It is to be seen if results obtained under laboratory conditions can be reproduced under field conditions. Experiments to study the conditions necessary for the rearing of the snakes in the snake-farm have been continued and it is noticed that the yield of the snake venom by snakes kept in the snake-farm is much greater than that of snakes kept in custody in the laboratory. Efforts made to rear the young snakes to adult stage in the snake-farms have so far not met with success. There is an indication to show that the snakes when kept in the farm live longer than when kept in custody in the cages. This latter observation will, however, require further study.

This year the demand for antirabic vaccine was even greater than last year. 8714 persons attended for treatment this year as against 7779 in 1952 and 6489 in 1951. One case of hydrophobia was reported from amongst those persons who were treated at the Institute Centre. The case was a multiple-bite case but yet the treatment was taken 45 days after exposure. The failure in this case thus obviously appears to be due to delayed treatment. Antirabic serum has now been prepared and if laboratory experiments confirm the superiority of the combined treatment of serum and vaccine over the single treatment with vaccine, it would be possible to use both serum and vaccine in so-called "bad" cases. The results of laboratory experiments are awaited.

The study on the isolation of influenza virus strains was continued and one A (Prime) strain was isolated from one of the suspected cases. Previously influenza B strains have been isolated. It thus appears all three strain A, A (prime) and B strains cause influenza infection in this country. An investigation undertaken with a view to standardise laboratory methods with the object of diagnosing atypical cases of small-pox from chicken-pox has now been completed and it would be possible in future to diagnose such cases definitely by laboratory methods within a short time. This investigation was originally undertaken to give help to Port Health Authorities in diagnosing suspected cases on board the steamer and with laboratory aid they would now be able to take appropriate measures in time.

The Department of Chemotherapy manufactured sulphathiazole tablets, powder and injectible, vitamin tablets, the hydnoceol oil and sulphetrone injectibles. The sale of both sulphathiazole tablets and vitamin tablets was higher this year than during the previous year. The sale was made to

Government institutions and hospitals, private medical practitioners and charitable institutions. 35,20,600 sulphathiazole tablets, 363 lbs. of B. P. powder and 19,500 millilitres of injectible sulphathiazole were sold during the year. About 95,00,000 of vitamin tablets of different composition were also sold during the year. There is scope for increasing the sales of these products and for some time the question of their sales through a well-established distributing agency was under consideration. Now that a Business Manager has been appointed he will be able to give attention to it, after he has completely studied the financial working of this Institute. Investigations into the manufacture of sulphamerazine, sulphamethazine etc., have been continued and research work is also being carried out to develop newer drugs of potential value in the treatment of malarial and bacterial infections.

The Department of Clinical Pathology continued the good work of giving diagnostic aid to various Government and Municipal Hospitals in the City and free diagnostic aid to private medical practitioners in case of infectious diseases. It also served as a reference laboratory for venereal diseases to City Hospitals and Laboratories by supplying standard diagnostic reagents and checking the accuracy of their results. There is a tendency on the part of certain city hospitals to send material for tests which are simple and can be easily carried out in the laboratories attached to their hospitals. Time taken for doing these simple tests prevents the Institute from doing work of more specialised nature and thus indirectly deprives the hospitals of aid in this respect. If heads of city hospitals pay attention to this aspect, this Institute will be able to give them more aid in highly specialised type of laboratory work.

The Blood Bank Department continued its normal activity of supplying dried plasma to hospitals and practitioners in the City and outside but the demand for this product was less than last year's. This may be due to the introduction in the market of synthetic plasma expanders like Dextran and P. V. P. which are freely available and whose cost is lower than that of plasma.

Besides the manufacturing activities and the research work carried out by regular members of the staff of the Institute, facilities were also provided to a number of full-time research workers to carry out research work under Fellowship offered by the State Government, Indian Council of Medical Research, National Institute of Sciences and other Institutions.

The total expenditure for the financial year 1952-53 was Rs. 21,01,642 and the income was Rs. 18,56,376.

III. Department of Vaccines.

During the year under review the demands for plague vaccine fell to still lower level than that of the last year while that for T. A. B. vaccine was also slightly lower. However, there was an exceptionally heavy demand for cholera vaccine during the year due to the outbreak of cholera epidemic all over the country. Cholera vaccine production had to be put to the maximum level for about four months to meet the demands for nearly 15 million millilitres of vaccine within a short space of time. A quantity

as great as 6·6 million millilitres was issued during the month of August alone. The demand for cholera vaccine supplied during the period was unprecedented in the history of this Institute. It is gratifying to note that such a heavy demand within such a short space has been successfully met. This has been possible, due to commendable efforts and devotion to duty shown by the staff in general, and particularly by the staff of the Vaccine Department, the Media Department and the Despatch Department.

1·1 million millilitres of plague vaccine, 19·8 million millilitres of cholera vaccine and 3,21,225 millilitres of T. A. B. vaccine were supplied as against 4·7 million millilitres of plague, 3·8 million millilitres of cholera and 4,10,754 millilitres of T. A. B. vaccine respectively in the year 1952. The total quantity of vaccines issued during the year 1953 is 21·3 million millilitres.

On the whole the demand for plague vaccine continued to show the same downward trend as in the last four years. The demand for cholera vaccine has shown such a steep rise after about five years. The demands for T. A. B. vaccine are more or less steady.

The demands for vaccines were received from all the 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 :—

Months	Quantity in millilitres		
January	...	...	29,185
February	...	...	4,19,425
March	...	...	2,24,310
April	...	...	13,750
May	...	...	1,748
June	...	...	11,675
July	...	...	26,180
August	...	...	255
September	...	...	20,450
October	...	...	1,60,530
November	...	...	1,93,812
December	...	...	75,412
Total ...			<u>11,76,727</u>

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

Year				Quantity in millilitres
1898-1901	...	...	...	86,01,123
1902-1906	...	...	...	1,03,66,611
1907-1911	...	...	...	1,07,52,786
1912-1916	...	...	...	1,37,02,098
1917-1921	...	...	...	2,00,03,682
1922-1926	...	...	...	2,16,07,908
1927-1931	...	...	...	1,77,87,729
1932-1936	...	...	...	1,99,09,176
1937-1941	...	...	...	2,00,78,811
1942-1946	...	...	...	4,45,28,208
1947-1951	...	...	...	6,57,57,991
1952-1953	...	...	...	59,29,507
Total ..				25,90,25,630

The plague vaccine is 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>			Quantities in millilitres
Bombay State	...	...	...	85,582
Uttar Pradesh	...	...	...	2,720
Madhya Pradesh	...	...	...	3,53,000
Vindhya Pradesh	...	...	...	26,500
Hyderabad (Deccan)	...	...	...	1,02,500
Madras State	...	...	...	30,225
West Bengal	...	...	...	1,52,000
Mysore	...	...	...	300
Delhi	...	...	...	490
Bihar	...	...	...	25,170
PEPSU	...	...	...	2,000
Punjab (East)	...	...	...	24,950
Travancore-Cochin	...	...	...	4,000
Defence Services	...	...	...	13,840
Total ...				8,23,227

Foreign countries.

				Quantities in millilitres.
Burma	...	...	...	3,50,000
Malaya	...	...	...	420
Afghanistan (WHO)	...	...	...	3,000
Gantak	...	...	...	30
Total ...				3,53,450

B. CHOLERA VACCINE.

During the year under report, the quantities of Cholera Vaccine despatched monthly are as given below :—

Months				Quantities in millilitres
January	...	...	...	1,48,993
February	...	...	...	58,860
March	...	...	...	3,14,605
April	...	...	...	1,78,019
May	...	...	...	9,83,136
June	...	...	...	17,58,742
July	...	...	...	29,92,157
August	...	...	...	66,27,255
September	...	...	...	36,49,889
October	...	...	...	13,91,525
November	...	...	...	13,99,525
December	...	...	...	3,28,833
Total				1,98,31,539

C. T.A.B. VACCINE.

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

Months				Quantities in millilitres
January	...	...	...	11,324
February	...	...	...	7,307
March	...	...	...	20,190
April	...	...	...	18,301
May	...	...	...	48,039
June	...	...	...	12,692
July	...	...	...	52,833
August	...	...	...	21,010
September	...	...	...	59,993
October	...	...	...	36,839
November	...	...	...	19,829
December	...	...	...	12,868
Total				3,21,225

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 the Institute,
and
- (3) The manufacture of parenteral solutions.

1. During the year 4,337 flasks of casein hydrolysate medium for plague and 31,663 flasks for cholera were prepared. For the preparation of T.A.B. Vaccine 1,295 roux bottles containing 200 ml. of agar in acid digest broth each were prepared.

2. Different types of media were prepared both for routine and research work of the Institute. For testing the sterility of vaccines, toxins, anti-toxine and parenteral solutions 88,000 bottles each of aerobic and anaerobic medium in acid digest broth were prepared.

3. The parenteral fluids section shows still further progress as may be seen from the following tables.

Sale of Glucose Saline etc. during 1953.

Serial No.	Name of Product.			Number of bottles sold.	Amount.
					Rs.
1	Glucose Saline	...	...	46,811	93,622
2	Hypertonic Saline	...	...	17,935	35,870
3	Normal Saline	...	...	6,870	13,740
4	Protein Hydrolysate	...	...	6,934	13,868
5	5 per cent. Glucose Solution	...	...	12,627	25,254
6	25 per cent. Glucose Solution 500 cc.	...	...	2,541	6,352
7	25 per cent. Glucose Solution 100 cc.	...	...	2,491	1,245
8	50 per cent. Glucose Solution 100 cc.	...	...	1,129	1,129
9	50 per cent. Glucose Solution 200 cc.	...	...	48	96
10	Sodium Sulphate	...	...	684	1,368
11	Sodium Citrate	...	...	925	1,850
12	Distilled Water	...	...	1,078	539
	Total	...	...	1,00,073	1,94,934

The corresponding figures of sales in the previous three years were as follows :—

1950	...	...	...	47,111	88,838
1951	...	...	...	54,892	1,03,232
1952	...	...	...	67,942	1,28,930

E. SECTION OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY.

This Section mainly examined samples of water for ascertaining their fitness for drinking, sanitary and industrial purposes. Bacteriological examination of some food samples was also undertaken.

A special reference may be made to the samples of water received from Nadiad, where 'epidemic dropsy' was prevalent. These samples, in addition to the routine chemical and bacteriological examinations, were subjected to special bacteriological examinations with a view to detect the presence of any pathogenic organisms. But no pathogenic organisms could be detected though the samples showed evidence of faecal pollution. The following table gives the summary of the various examinations carried out during the year.

Sample examined.	Nature of examination.			No. of samples examined.
Water	...	Fitness for drinking purposes	...	82
Water	...	Fintess for bathing	...	4
Water	...	Fitness for sanitary and industrial purposes.	...	36
Gripewater	...	Bacteriological examination	...	1
Milk powder	...	Do.	...	2
Canned potatoes.	...	Do.	...	3
Meat	...	Do.	...	1
Total				129

IV. Department of Antitoxins and Sera.

The year under review was important from the point of view of expansion of the activities of the department. For the last few years the demands for antitoxins and sera from the State as well as from other States were on the increase. The Armed Forces had also approached us for the supply of their needs of sera, particularly of Antivenene and Tetanus Antitoxins. With the resources of men and material available it had not been possible to meet all demands made for our sera in the past. Hence, with a view to expand the production work, a stud farm, as reported previously, was taken on hire basis

to house a reserve of horses for preliminary immunization. This enabled us to use all available space at the Institute stables for housing the horses for hyper immunization. This has resulted in increased production even over that of the last year. The farm has now been permanently acquired for the department and if all the demands made on the Institute are to be met, further expansion of the activity of the stud-farm will have to be undertaken.

Production of Antitoxins and Sera even exceeded the highest level of the last several years. During the year a total of 14,08,100 ml. of both concentrated and natural Antitoxins and Sera were produced, as against 842,500 ml. of 1952, which incidentally was the highest level of production so far. The production and supply of Anti-snake-venom Serum during the year was almost double of the average of the last few years; similarly the supply of Tetanus Antitoxins was also very greatly increased. Further increase in production will depend on the development of the Pimpri stud-farm.

At the request of World Health Organization an attempt was made to produce Antirabic Serum in horses and six horses were immunized. The preliminary tests undertaken to assess the protection value of the sera in mice have yielded satisfactory results and it is hoped that a potent Antirabic Serum will soon be made available for experimental and clinical trials.

There has been a further drop in the demands for Anti-dysentery and Anti-plague sera. The newer remedies for dysenteries such as antibiotics are now in use, and this holds good for treatment of plague, the incidence of which is consistently falling in the last few years.

1. *Horses*.—Total number of horses used during the year for production of antitoxins and sera was 185. Besides these, there were 53 horses at Pimpri under preliminary immunization. The horses were grouped as follows :—

Antitoxins				Number of horses used.
1. Tetanus antitoxin	...	...	...	98
2. Diphtheria antitoxin	...	...	...	42
3. Anti-snake-venom Serum	...	...	...	29
4. Welchii antitoxin	...	...	...	6
5. Antirabic	...	...	...	6
6. Dysentery	...	...	...	2
7. Preliminary Immunization	...	...	...	53
8. Normal Serum		...	...	2
Total				238

2. *Preparation of Toxins and Toxoids.*—During the year, diphtheria, tetanus and welchii toxins were prepared for hyper immunization of horses. The reserve stock of the other toxins sufficed for the needs of the year. A part of the diphtheria and tetanus toxins were converted into toxoids for immunization of horses. A portion of these toxoids was processed for human use.

Types of Toxins and Toxoids.					Quantity in Litres.
1. Tetanus	...	...	...	...	502
2. Diphtheria	...	...	...	...	222
3. Welchii	...	...	...	...	49
4. Septique	...	...	...	...	nil.
5. Oedematiens	...	...	...	...	nil.
6. Shiga	...	...	...	...	nil.
7. Tetanus toxin converted to toxoid	...	...	...	...	51
8. Diphtheria toxin converted to toxoid	...	...	...	...	140

3. *Preparation of Anti-toxins and Sera.*—During the year a total of 1,39,000 ccs. of concentrated sera was produced from 8,023,000 ccs. of plasma. This quantity is about 65 per cent. more than the previous year and is more than double the average production in the previous years. The quantity of each type of sera is as follows:—

I. CONCENTRATED SERA.

No.	Name.	Amount of concentrated serum in ml.
1. Tetanus Antitoxin		8,92,200
2. Diphtheria Antitoxin		1,84,300
3. Gas Gangrene		69,200
4. Antivenene		2,35,000
5. Anti-dysentery		10,300
Total		13,91,000

II. NATURAL SERA.

No.	Name:	Amount of concentrated serum in ml.
1. Normal Horse Serum		17,100

4. *Lyophilisation of Sera.*—Only the polyvalent anti-snake-venom serum was issued in lyophilised form during the year. The amount of serum issued was 1,05,580 ml.

5. *Supply*.—The sale value of products during the year was Rs. 6,77,901 which is about 24 per cent. increase over the previous year and an increase of about 90 per cent. over the average of previous years. The following quantities of antitoxins, sera and toxoids were issued during the year under review. In addition to the list of items shown in the table below, 16,100 ml. of Natural Horse Serum was supplied to local hospitals.

Name of Sera.	Quantity in ml.
1. <i>Tetanus Antitoxin</i> .—	
(a) Prophylactic 3,000 I. U. in 2 ml. ...	4,78,108
(b) Curative 10,000 I. U. in 5 ml. ...	17,765
(c) Curative 20,000 I. U. in 10 ml. ...	1,06,870
(d) Highly concentrated 20,000 I. U. in 4 ml.	67,768
2. <i>Diphtheria Antitoxin</i> .—	
(a) Prophylactic 1,000 I. U. in 2 ml. ...	2,684
(b) Curative 5,000 I. U. in 5 ml. ...	4,000
(c) Curative 10,000 I. U. in 10 ml. ...	1,39,700
(d) Highly concentrated 10,000 I. U. in 5 ml ...	23,205
3. <i>Gas-Gangrene Antitoxin</i> .—	
(a) 3,000 I. U. Welchii Antitoxin in 10 ml. ...	69,470
(b) 9,000 I. U. Welchii, 4,500 I. U. Septique and 9,000 I. U. Oedematiens Antitoxins, in a volume not exceeding 15 ml., highly concentrated and purified.	1,740
4. <i>Anti-plague serum, 20 ml.</i> —	500
5. <i>Lyophilised Polyvalent Anti-snake-venom Serum 10 ml.</i>	1,05,580
6. <i>Anti-dysentery Serum</i> .—	
(a) Natural, 20 ml. ...	809
(b) Concentrated 4,000 I. U. in 5 ml. ...	265
7. <i>Tetanus Toxoid</i> .—	
(a) 10 ml. ...	1,820
(b) 5 ml. ...	1,505
(c) 1 ml. ...	1,412
8. <i>Diphtheria N. A. F. T. Toxoid</i> .—	
(a) 5 ml. ...	535
(b) 1 ml. ...	660
9. <i>Lyophilised Schick Test Toxin for 25 Tests</i> .—	4 (boxes)
10. <i>Normal Horse Serum 10 ml.</i>	1,880
Total: 10,20,335 ml. of antitoxins and sera, and 5,932 ml. of toxoids.	

V. Department of Entomology.

During the year technical assistance was given to health authorities of different States of the Indian Union, in connection with the information on Plague work, with particular reference to Fleas, Rats, Rat traps, Pulicides, and Rodenticides. Several enquiries from Institutions and private parties were attended to, in connection with samples of 'WORDER-TRAP', anti-coagulant rodenticides, rat destruction and arachnid bites.

Examination of Samples.—80 samples of water and 20 samples of milk were examined microscopically for animacules. 20 flea pooling experiments were done for the Public Health Department of the State with a view to detect the presenece or absence of plague infection in them by using fleas recovered from different areas in the Bombay State. All these experiments were negative to Plague.

Classes.—Plague Preventive Measure Classes were conducted from the 6th April to 21st April 1953. The total number of students that attended the classes were 18. Seven of these were from Bombay Municipality, 7 from the Department of Public Health, Poona, and 1 each from Miraj, Bijapur, Gokak and Athani Municipalities.

A. SNAKES.

Twenty-seven snake specimens sent by hospitals and other private parties with history of snake-bite cases, were identified.

Experiments done during the year have enabled this Institute to start an open air snake-farm in the compound. Here three kinds of poisonous snakes, i.e. Cobra, Krait and Russels Viper are being kept in natural surroundings, most suited to their habits. The average venom yield per snake kept in the farm has been seen for the last 12 months to be greater than that of snakes kept in custody in cages.

Average venom yield per snake for the 12 months of 1953 in gms.

Cobra.		Krait		R. Viper.	
Farm.	Room.	Farm.	Room.	Farm.	Room.
0·2117	0·1186	0·02207	0·01523	0·1519	0·1015

The production and supply of different venoms in gms. during the year is as follows :—

	Cobra	Krait	R. Viper	Saw-scaled. Viper.
Production	70·98	11·25	73·88	7·95
Supply	30·45	4·05	30·95	2·05

A scheme financed by the Indian Council of Medical Research for the studies on 'Indian Poisonous Snakes' was continued. Ecological and morphological studies on Cobra, Krait, Russel's Viper and Echis were conducted during the year along with the biochemical studies on inhibitors, if any, in these snake sera.

An Enquiry was conducted during the year on the Entomological Survey of the Swadeshi Mills area, Kurla. About 28,127 insects belonging to 50 different families have been collected. A number of these insects are of medical importance and are found breeding in the area. Some of these have been identified by the British Museum (Natural History), London.

Studies were carried out on the free living flea ectoparasites of *R. rattus* and *G. kok* during the year. From the total 815 rats examined, 1,289 fleas were recovered. Of the total number of fleas recovered, 984 were *X. cheopis* and 305 *X. astia*. 666 *X. cheopis* were from *R. rattus* and 318 from *G. kok*, while 82 *X. astia* were from *R. rattus* and 223 from *G. kok*. These studies are being conducted to see if there is any correlation between different species of fleas on different rats with their degree of susceptibility to plague.

The maximum number of days required for the completion of the life history of *X. cheopis* in the laboratory were 41 during the month of December and minimum number of days for the completion of cycle were 20 in the month of May 1953. Work has just been started on the comparative study of the different rats of Bombay with particular reference to their acarine parasites.

Pulicidal Value of Pyrethrum.—91 preliminary experiments done during the year have shown that the powder formulations of Pyrethrum extract mixed with a booster Piperonyl Butoxide gave a residual effect lasting upto 336 days.

B. EXAMINATION OF BOMBAY CITY RATS.

(a) During the year under review, the City was absolutely free from human plague infection. But still, to be able to assess the vulnerability of different spots in the City to plague infection, we undertook the study of determining the flea index of various wards. For this purpose, rats were trapped from different places like grain godowns, heavily congested chawls and other places which are known to be harbouring rats in great numbers. The work was started in July when the season becomes favourable for flea breeding and consequently for the spread of the epizootic among the rats. The wards were given priority based on the number of plague infected rats found in that ward during the last 5 years and also on the average flea index of the ward as estimated last year. The ward which was supposed to be most vulnerable was taken first for our study and the least vulnerable last. In all

129 flea counts were carried out and out of these 52 gave a zero index. The following is a ward-wise summary of the results :—

Ward	No. of flea counts carried out	No. of flea counts with zero index.	Average flea index
G-North	29	10	1.6
E-East	24	8	2.1
C	21	9	0.1
G-South	10	6	2.5
F	29	8	0.5
A	9	5	0.6
B	7	6	0.1

Though on the whole the results are very satisfactory we did come across a few places which showed a flea index as high as 8. The study has proved useful in as much as it gives at a glance a correct idea about the vulnerability of different places to plague infection in the City of Bombay.

(b) It is known that flea-pool inoculation gives very valuable information about the seat of infection. Sometimes positive results are obtained even when we fail to detect the infection in the rodents. Therefore in order to make the search for plague infection fully effective, flea-pools collected from rats trapped from different wards were crushed and inoculated into white mice, as this animal is known to be highly susceptible to plague infection. In all 129 flea-pool inoculations were carried out. It is very gratifying to note that the results in all cases were negative.

(c) Experiments on the susceptibility of the 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 the experiments.	No. of rats dead.	Mortality per cent.
R. rattus	80	11	13.7
R. norvegicus	69	4	5.8
Gunomys kok	80	66	82.5

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

C. EXAMINATION OF RATS AND FLEAS FROM STEAMERS AND OTHER SOURCES.

During the year 1953, 9 dead rats, 41 smears of dead rats and 1004 fleas were received from different towns of the Bombay State. In none of them plague infection was detected. In the same year, 303 killed rats were received from the Health Officer of the Port of Bombay. Of these, 237 belonged to the species *Rattus rattus refescens*, 14 *Rattus norvegicus*, 32 *Mus dubius* and 20 Putrid. None of them was found plague infected.

24 rats were also received from the City of Bombay through other agencies or private persons. None of them was found to be plague infected.

TABLE I

Showing rats received at the Institute from the Bombay Municipality during 1953.

Month.	Alive.	Dead.	Total.
January	1,04,651	234	1,04,885
February	85,905	360	86,265
March	90,405	317	90,722
April	84,201	234	84,435
May	85,291	73	85,364
June	80,058	178	80,236
July	83,834	630	84,464
August	82,200	342	82,542
September	75,960	141	76,101
October	84,155	86	84,241
November	83,459	73	83,532
December	92,473	51	92,524
Total	10,32,592	2,719	10,35,311

TABLE II.

Showing dead rats examined for plague during 1953.

Month.	Dead rats examined.	Found plagues infected.
January	207	Nil.
February	328	Nil.
March	315	Nil.
April	218	Nil.
May	62	Nil.
June	156	Nil.
July	609	Nil.
August	311	Nil.
September	129	Nil.
October	67	Nil.
November	51	Nil.
December	37	Nil.
Total	2,490	Nil.

In addition to dead rats, 19 live rats from plague suspected localities and 6,642 live rats from the Bombay Port Trust area were examined during the year. The total number of live and dead rats examined is 9,151.

TABLE III.

Showing the number and percentage of different species of rats trapped alive by Municipal Corporation during 1953.

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i>	.. 27,167	25·9	19,525	22·7	18,058	20·0	16,744	19·9	18,450	21·6	17,845	22·3
<i>Rattus norvegicus</i>	.. 18,700	17·9	15,697	18·3	16,494	18·3	14,745	17·5	14,702	17·3	13,353	16·7
<i>Gunomys kok</i>	.. 38,282	36·6	33,264	38·7	37,676	41·7	35,148	41·7	34,723	40·7	31,945	39·9
<i>Bandicota malabarica</i>	.. 1,040	1·0	891	1·1	668	0·7	676	0·8	566	0·7	785	1·0
<i>Mus dubius</i>	.. 7,146	6·8	5,354	6·2	5,453	6·0	5,274	6·3	5,481	6·4	4,698	5·8
<i>Suncus caeruleus</i>	.. 12,316	11·8	11,174	13·0	12,056	13·3	11,614	13·8	11,369	13·3	11,432	14·3
Total	.. 1,04,651	100·0	85,905	100·0	90,405	100·0	84,201	100·0	85,291	100·0	80,058	100·0

TABLE III—*contd.*

Species.	July		August		September		October		November		Decmeber		Whole year.	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i>	.. 19,926	23·8	21,247	25·8	17,929	23·6	22,219	26·4	23,194	27·8	24,809	26·8	2,47,113	23·9
<i>Rattus norvegicus</i>	.. 14,216	17·0	14,640	17·8	13,233	17·5	14,681	17·4	13,175	15·8	16,279	17·6	1,79,965	17·4
<i>Gunomys kok</i>	.. 31,570	37·7	28,483	34·7	27,472	36·2	27,160	32·3	26,645	31·9	30,429	32·9	3,82,797	37·1
<i>Bandicota malabarica</i>	.. 718	0·8	662	0·8	548	0·7	721	0·9	738	0·9	888	1·0	8,901	0·9
<i>Mus dubius</i>	.. 4,571	5·4	5,019	6·1	5,633	7·4	6,141	7·3	6,854	8·2	7,651	8·3	69,275	6·7
<i>Suncus caeruleus</i>	.. 12,833	15·3	12,149	14·8	11,095	14·6	13,233	15·7	12,853	15·4	12,417	13·4	1,44,541	14·0
Total	.. 83,834	100·0	82,200	100·0	75,960	100·0	84,155	100·0	83,459	100·0	92,473	100·0	10,32,592	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 1953.

Species.	January		February		March		April		May		June		
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	
<i>Rattus rattus rufescens</i>	..	105	44.9	202	56.1	146	46.1	81	34.6	26	35.6	59	33.2
<i>Rattus norvegicus</i>	..	40	17.1	53	14.7	70	22.1	46	19.6	12	16.4	46	25.8
<i>Gunomys kok</i>	..	59	25.2	71	19.7	99	31.2	89	38.2	23	31.5	47	26.4
<i>Bandicota malabarica</i>	..			1	0.3			1	0.4			4	2.3
<i>Mus dubius</i>	..	3	1.3	1	0.3			1	0.4	1	1.4		
<i>Suncus caeruleus</i>	..											1	0.5
Putrid	..	27	11.5	32	8.9	2	0.6	16	6.8	11	15.1	21	11.8
Total	..	234	100.0	360	100.0	317	100.0	234	100.0	73	100.0	178	100.0

TABLE IV—*contd.*

	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>	.. 359	57.0	168	49.1	58	41.1	34	39.5	32	43.9	19	37.3	1,289	47.4
<i>Rattus norvegicus</i>	.. 127	20.2	78	22.8	32	22.7	10	11.6	6	8.2	9	17.6	529	19.3
<i>Gunomys kok</i>	.. 117	18.6	64	18.7	37	26.3	22	25.6	13	17.8	9	17.6	650	23.9
<i>Bandicota malabarica</i>	.. 2	0.3	1	0.3	2	1.4	1	1.2					12	0.5
<i>Mus dubius</i>	.. 4	0.6											10	0.4
<i>Suncus caeruleus</i>	.. 1	0.1											2	0.1
Putrid	.. 20	3.2	31	9.1	12	8.5	19	22.1	22	30.1	14	27.5	227	8.4
Total	.. 630	100.0	342	100.0	141	100.0	86	100.0	73	100.0	51	100.0	2,719	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 1953.

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ...	324	48.5	197	38.0	286	48.4	361	53.3	236	43.1	390	52.7
<i>Rattus norvegicus</i> ...	55	8.2	79	15.2	46	7.8	64	9.5	35	6.4	49	6.6
<i>Gunomys kok</i> ...	119	17.8	69	13.3	88	14.9	113	16.7	58	10.6	45	6.1
<i>Bandicota malabarica</i> ...	3	0.5	1	0.1	10	1.7	3	0.4	3	0.5	12	1.6
<i>Mus dubius</i> ...	85	12.7	111	21.4	105	17.7	112	16.5	183	33.4	178	24.1
<i>Suncus caeruleus</i> ...	82	12.3	62	12.0	56	9.5	24	3.6	33	6.0	66	8.9
Total ...	668	100.0	519	100.0	591	100.0	677	100.0	548	100.0	740	100.0

TABLE V—*contd.*

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> ...	333	65.4	305	56.4	253	49.0	443	62.0	450	63.9	381	51.8	3,959	53.1
<i>Rattus norvegicus</i> ...	49	9.6	37	6.8	17	3.3	31	4.3	60	8.5	107	14.5	629	8.4
<i>Gunomys kok</i> ...	45	8.9	42	7.8	108	20.9	106	14.9	53	7.5	100	13.6	946	12.7
<i>Bandicota malabarica</i> ...	1	0.2	7	1.3	4	0.8	12	1.7	11	1.6	3	0.4	70	0.9
<i>Mus dubius</i> ...	52	10.2	105	19.4	117	22.7	90	12.6	92	13.1	77	10.5	1,307	17.5
<i>Suncus caeruleus</i> ...	29	5.7	45	8.3	17	3.3	32	4.5	38	5.4	68	9.2	552	7.4
Total ...	509	100.0	541	100.0	516	100.0	714	100.0	704	100.0	736	100.0	7,463	100.0

TABLE VI.

Showing the different localities in the Bombay Province from which smears, rats and fleas were received for examination during 1953.

Name of Town				No. of smears.	No. of rats.	Fleas	
						No. of sampels	No. of fleas.
Revdanda	...	...	...	2	...	...	...
Bassein	...	...	...	1	1	...	...
Islampur	...	...	...	4	...	...	...
Ahmednagar	...	...	...	1	...	...	...
Karad	...	...	...	6	...	...	...
Chikodi	...	...	...	6	...	...	...
Thana	...	...	...	...	5	...	...
Brjapur	...	...	...	3	...	...	...
Bilimora	...	...	...	...	3	...	...
Sholapur	...	...	...	9	...	...	...
Sholapur (Andevadi Village)	...	...	...	1	...	2	101
Sholapur (Shawal Village).	...	...	...	...	...	2	109
Satara (Jakatvadi)	...	...	...	...	...	3	369
Baramati	...	...	...	...	...	2	84
Vengurla	...	...	...	...	...	6	249
Savantvadi	...	...	...	8	...	5	92
				41	9	20	1,004

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 1953 —

Name of the Institution		Number of rats supplied.	Name of the Institution.		Number of rats supplied.
1.	Wilson College	574	8.	Sidharth College	8
2.	Institute of Science	287	9.	Topiwala National Medical College.	21
3.	Jai Hind College	82	10.	Khalsa College	8
4.	Ramnarain, Rula College	169	11.	St Xaviers College	48
5.	Natural History Section, P. W. Museum.	2	12.	National College, Bandra	12
6.	Seth G. S. Medical College	6	13.	Elphinstone College	4
7.	Ruparel College	32			
Total No		...			1,253

VI. Department of Clinical Pathology and Diagnostic Reagents.

The Department of Clinical Pathology and Diagnostic Reagents continued to (1) conduct bacteriological, serological, microscopic and biological tests on clinical materials received 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 report on pathological tissues;

(4) prepare autogenous vaccines ; (5) conduct special tests such as the Rideal Walker Test, tests for pregnancy ; (6) train science graduates and others for laboratory duties ; (7) afford facilities for post-graduate research students ; and (8) conduct research.

1. General Bacteriological Work.

Clinical material from 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, cerebro-spinal fever, enteric group of fevers, typhus, plague, cholera, etc., were carried out free of charge.

(i) *The Government of Bombay Typhoid Scheme.*—Was continued for the sixth year and found very popular, and useful. 765 calls were attended to. The fall in the number of calls during the year under review was due to not attending to calls wherein Chloromycetin had already been administered for more than two days. Blood culture examination in such cases was considered futile. 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 positive cases 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 765 blood samples for culture and widal, 262, i. e., 34 per cent., were positive. Lesser number of samples for Widal and blood culture examination were received from Arthur Road Hospital as the hospital's own laboratory started to function, towards the later part of the year under review.

An analysis of records from this section showed the following interesting points :—

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

(b) *Results of blood culture* and triple widal test of samples examined during 1951, 1952 and 1953 are tabulated 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 cultures done	B. typho- sus iso- lated	B. paraz A isolated
1951 ..	2,200	1,436	764	141	505	154	4,151	487	71
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

(ii) *Venereal Disease Reference Laboratory*.—This department undertook to act, in 1951, 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. In all 928 samples were tested. Of these, there was agreement with our results to the extent of 96·5 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 in this Institute.

(iii) *Routine Examination*.—The number of routine examinations carried out during the years 1951, 1952 and 1953 are shown in the following table for purposes of comparison.

	1951	1952	1953
I. Serological Examinations—			
1. Wassermann Test ..	4,536	3,661	3,004
2. Kahn Test ..	2,589	2,627	2,935
3. Triple Widal Test ..	2,167	2,687	2,568
4. Agglutination Test for—			
(a) Typhus ..	4,206	3,142	2,568
(b) Brucellosis ..	2,186	2,811	2,573
(c) Leptospirosis ..	37	64	144
(d) Paul-Bunnell Test ..	10	19	9
II. Bacteriological and Biological Tests—			
1. Blood culture for enteric group ...	4,151	4,189	3,385
2. Urine culture for pathogenic organisms ...	113	67	123
3. Throat swabs for diphtheria ...	189	238	281
4. Stools and rectal swabs for culture ...	809	1,152	1,680
5. Sputum and body fluids for AFB ...	671	733	961
6. C. S. F. for culture ...	414	461	425
7. Materials from suspected plague cases ...	11	14	Nil.
8. Material for Medico-legal cases ...	27	33	25
III. General Microscopic Examinations—			
1. Blood films for M. P. and Filariasis ...	409	54	25
2. Blood cell count ...	1,363	1,621	1,942
3. Bone Marrow ...	22	6	26
4. Semen for fertility ...	50	56	43
5. Material for smear examination (presence for pathogenic Organisms Like G. C. etc.).	56	51	55
6. Stool—Routine test ...	1,057	1,068	1,019
7. Urine—Routine test ...	978	890	1,114

			1951	1952	1953
IV. <i>Pregnancy Test</i> —	...	...	386	523	691
V. <i>Rideal Walker Test</i> —	...	...	30	47	68

(iv) *Autogenous Vaccines*.—Use of autogenous vaccines is still in vogue but to a less 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 1951, 1952 and 1953 are shown below :—

		1951	1952	1953
Courses of autogenous vaccines supplied	...	56	55	28

2. *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. The demands for these reagents are progressively increasing in most commonly used items as may be seen from the following annual supply for the last three years.

<i>Reagents—</i>	<i>Quantity supplied in ml.</i>		
	1951	1952	1953
1. Killed suspensions for Widal and Weil—			
Felix tests	119,920	160,485	164,895
2. Blood Grouping Sera	5,719	7,170	7,776
3. High Titre Sera and Amboceptor	729	756	686
4. Kahn Antigen	3,964	5,825	4,932
5. W. R. Antigen	650	350	180
6. Russell's Viper and Cobra Venom solutions	2,313	2,893	1,436

(ii) *Supply of Stains*.—In addition to diagnostic reagents, the supply of various biological stains was continued and a total of 113,960 ml. of different stains were issued to different state and private laboratories. An increase of 7,530 ml. over the previous year is stressed.

(iii) *Collection of standard strains*.—Attempts have been made for collection of standard strains of different pathogenic micro-organisms with a view to meeting the growing demands for these strains. It is hoped to have a collection of standard strains in this laboratory in course of time.

3. Histopathology Section.

Pathological Specimens.—During the year under review, 2,056 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 brain tissues from suspected rabid animals, neoplasms, gynaecological conditions, tuberculosis of different organs and some rare and interesting pathological specimens. In addition, 219 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, 2,375 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 1951, 1952 and 1953 is tabulated below :—

		1951	1952	1953
<i>Classification.</i>				
1. Number of samples found to be normal	...	143	284	265
2. Number of samples unfit for examination	...	107	136	157
3. Brain Samples for Negri bodies	...	52	79	83
4. Samples for miscellaneous pathological conditions.	...	609	884	1,172
5. Samples for tuberculous infection	...	74	123	138
6. Non-malignant Neoplasms	...	74	65	95
7. Malignant Tumours	...	92	114	146
Total	...	1,151	1,685	2,056

It will be seen that there has been a steady and marked 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	...	...	...	24
Anaplastic Carcinoma	...	...	...	2
Adnexal Carcinoma	...	...	...	2
Basal Cell Carcinoma	...	...	...	1
Epidermoid Carcinoma	...	...	...	28
Embryonal Carcinoma	...	...	...	1
Medullary Carcinoma	...	...	...	4
Papillary Carcinoma	...	...	...	1
Scirrhus Carcinoma	...	...	...	1
Squamous Cell Carcinoma	...	...	...	2
Carcinoma Simplex	...	...	...	32

(2) Carcinoma Secondary—	...	...	...	16
(3) Sarcoma—	...	...	...	
Fibrosarcoma	...	...	...	9
Melanotic Sarcoma	...	...	...	3
Myeloma	...	...	...	1
Myosarcoma	...	...	...	1
Giant Cell Sarcoma	...	...	...	1
Rhabdomyosarcoma	...	...	...	2
Round Cell Sarcoma	...	...	...	1
Sarcoma	...	...	...	5
(4) Sarcoma Secondary	...	...	...	1
(5) Malignant Lymphoma Hodgekin's Sarcoma	...	...	...	2
Lymphosarcoma	...	...	...	2
(6) Teratoma	...	...	...	3
(7) Haemangio Endothelioma	...	...	...	1
<i>(b) Non-malignant Neoplasms :</i>				
Adenoma	...	...	...	2
Condyloma	...	...	...	2
Chondroma	...	...	...	1
Colloid Goitre	...	...	...	3
Diffuse Goitre	...	...	...	1
Cyst Adenoma	...	...	...	3
Fibroadenoma	...	...	...	17
Fibrolipoma	...	...	...	1
Fibroma	...	...	...	4
Fibromyoma	...	...	...	23
Fibroma Cellular	...	...	...	6
Granuloma	...	...	...	1
Haemangioma	...	...	...	2
Haemangioma Capillary	...	...	...	2
Mixed Parotid Tumour	...	...	...	1
Mycetoma	...	...	...	1
Myxoma	...	...	...	1
Neurofibroma	...	...	...	6
Osteoma	...	...	...	1
Osteochondroma	...	...	...	1
Osteoclastoma	...	...	...	2
Papilloma	...	...	...	7
Papilliferous Cyst Adenoma	...	...	...	1
Pseudomucinous Cystadenoma	...	...	...	2
Rhinoscleroma	...	...	...	1

II. TUBERCULOSIS IN DIFFERENT ORGANS.

Breast	...	...	...	2
Female Genital Tract	...	...	...	71
Lymphnodes	...	...	...	32
Intestinal Tract	...	...	...	1
Other Tissues	...	...	...	32

An interesting specimen received during the year was specimen No. 660 of 2nd May 1953. From Labia Major. Sections of the growth showed appearances of a normal testicle. Full clinical details were obtained and showed that it was a case of female of 14 years of age, who had a fully developed testicle with epididymis, vas, etc., on the right side of external genitals.

4. *Special Investigations.*

During the year under review, the department was entrusted with investigations in connection with the following epidemics :

(i) *Epidemic of Polyuria and Polydypsia in Bota near Poona.*—Col. J. R. Dogra went to see the cases at Bota on 27th February 1953 and to collect samples of blood, urine, stools and drinking water with a view to isolating of an infective agent, if any. These investigations showed negative results. The disease which appeared to be one that affected all ages, both sexes, and showed seasonal prevalence, was curable with hospitalization and caused no mortality.

(ii) *Mysterious Disease (Epidemic dropsy) prevailing in Nadiad.*—Soon after the outbreak of a "mysterious disease (Epidemic Oedema Syndrome)" in Nadiad (District Kaira) in the middle of June 1953, Dr. B. G. Modi, in collaboration with Dr. V. N. Jai of the Department of Nutrition, Government of Bombay visited Nadiad to study the epidemiological and clinical features of the disease on the spot, and brought samples of blood, urine, stools, drinking water, cereals and edible oils for investigation.

Clinical examination showed that people of all socio-economic levels experienced malaise, vague upper abdominal discomforts, passed frequent and semi-liquid stools and developed oedema of the feet. The latter extended, in the majority of the cases, up to the knee-joints. In addition, an erythematous rash, particularly over the oedematous parts was noticed. The clinical picture resembled 'epidemic dropsy' including myocardial damage, in severe cases.

Routine and cultural examinations of blood and urine did not indicate any bacterial aetiological factor. Cultural examination of stools showed organisms of *Proteus* and *Paracolon* group only.

Subsequent investigations of samples of edible oils, etc., showed the presence of argemone oil in some of the oil samples mainly *groundnut oil*.

The question of water-borne infection as an aetiological factor was also investigated. Samples of water showed evidence of faecal pollution. The disease is on the decline since August 1953. A few deaths have been recorded.

(iii) *Outbreak of Cholera in Mental Hospital, Thana.*—In the second week of September some cases of diarrhoea and vomiting occurred in the Mental Hospital, Thana. Col. J. R. Dogra visited the hospital for investigation. He found the cases to be those of cholera, clinically. This was subsequently confirmed by cultural examination of stools and vomitus from which *V. cholera* was isolated. Preventive measures suggested by him were carried out by the hospital authorities and the spread of cholera was checked.

(iv) *Epidemic Jaundice in Sassoon Mills, Bombay.*—During the months of November and December 1953, an epidemic of jaundice broke out among the staff of the Sassoon Mills, Mazagaon. During the period of about three weeks about 800–1000 cases were reported.

Some of these cases were admitted to the Arthur Road Hospital for detailed investigations.

Routine, cultural and biochemical examinations of blood, urine and stool were carried out in a representative group of 28 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 process of elimination suggested the possibility of infectious hepatitis. No mortality was reported.

Attempts at isolation of a virus were unsuccessful.

(v) *Inter-laboratory Evaluation of Serologic Tests on Syphilis.*—This department agreed to co-operate in this enquiry of the Indian Council of Medical Research. Starting in June 1953, 397 samples of sera were obtained in 30 batches. Each batch of sera was subjected to quantitative and qualitative Wassermann, Kahn and V. D. R. L. tests. This involved a great deal of work.

5. *Training and Research Scholars.*

The following research scholar worked in the Department :—

Shri S. M. Vaidya with effect from January 1953, 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. Makhanlal Jain : Paying Trainee from 2nd March 1953 to 31st March 1953.
2. Shri M. M. Deshpande : Paying Trainee from 17th September 1953 to 30th November 1953.

Research.

6. Practically every senior member of the staff conducted research work alone or in collaboration with others. For further details see Part II of this report.

VII. Department of Pharmacology.

During the year under review the activities of the Department increased considerably than that of the preceding year. It met the increasing demand for testing of drugs and carried out investigations on problems of importance arising out of the work. The pressure of routine work was very heavy and consequently the members of the staff could spend only a small part of their time on research problems.

Several problems of interest were investigated. The efficiency of protein hydrolysates in maintaining nitrogen balance in patients by intravenous medication essentially depends on the amino acid contents of such preparations. An investigation showed that the Casein Hydrolysate prepared at this Institute contained all the essential and non-essential amino acids in reasonable proportions.

Study on the stability of Vitamin B₁, demonstrated that the potency of the vitamin is completely lost at room temperature in course of one year when combined with crude (oral) liver extracts and vitamin A. The greater the concentration of the liver extract, the more marked is the loss of potency.

A general survey of the results of estimation of Folic Acid in complex multivitamin preparations revealed that the vitamin did not remain stable in some of these preparations.

4 : 4.—diamino diphenyl sulphone (DDS), a drug effective against leprosy has been shown to traverse from maternal to the foetal circulation in the rabbit but it has no adverse effect on the gravidity. This observation should be confirmed in human beings as the duration of pregnancy is not only longer but the treatment with DDS may extend for a long period.

In all, 1,909 samples were examined during the year out of which 1213 were under the Drugs Act, 1940, and the Rules thereunder, and the rest, i.e. 696 were received from other sources which included our Institute, manufacturing firms, Government departments, hospitals etc.

The following table gives the comparative figures of the total number of samples tested during the last five years :

	1949	1950	1951	1952	1953
No. of samples tested ...	508	924	1,284	1,524	1,909

The above table shows that the present work is about four times that of 1949 and nearly double that of 1950.

There has been persistent increase in the demand for testing of "biological and special products" and "other special products" during recent years. In 1951, 53 per cent. of the total samples examined were under this category but this figure rose to 64 per cent. in 1952 and to 70 per cent. in the year under review.

Out of the 1,213 samples tested under the Drugs Act, 800 were received from the Bombay State, 146 from East Punjab, 117 from Delhi, 91 from Uttar Pradesh, 51 from Madhya Pradesh, 5 from Bihar, 2 from Saurashtra and 1 from Assam.

In 1953, about 27·0 per cent. of the samples were of non-standard quality as against 33 per cent. recorded during the previous year. This improvement in the quality of the drugs was observed in the preparations which were sent here for "quality control". On the other hand, the quality of the drugs that were tested under the Drugs Act did not show any change for the better. Under this category 1,213 samples were examined out of which 425 were of non-standard quality, i.e. about 35 per cent. of the total. This does not compare favourably with the figure of 1952 which is 37 per cent.

It is rather depressing to note that there exists a group of unscrupulous persons who would not hesitate to substitute powdered Sulphanilamide for Procaine Penicillin, Starch, Chalk or Kaolin for Quinine Ethyl Carbonate (tasteless quinine), Sodium Bicarbonate for Chloromycetin, and a yellow powder for Aureomycin. The imitations of the samples were so good that it would have been difficult for lay public to distinguish them from genuine ones. These are some of the glaring instances of faking of essential life-saving remedies. It is hoped that with the co-operation of medical men and those who are in the pharmacy trade, profession and industry and with the application of more stringent Drug Control measures it would be possible not only to improve the quality of the drugs in our country but also to detect the persons who are dealing in spurious medicines.

The samples cover a wide variety of drugs, both pharmacopoeial and non-pharmacopoeial preparations, essential and edible oils, synthetic chemotherapeutics, hormones, vitamins, alkaloidal preparations, patent and proprietary medicines, insecticides, surgical dressings, glass containers etc.

The large number of drugs examined and the diversity of their nature do not permit us in view of the brevity of the space, a detailed discussion of the results of examination. It is also not possible to cite in every case the reason for which a drug has been declared to be of non-standard quality. However, some general observations are presented here for the information of those who are interested in drug testing.

Examination of drugs by chemical means.—Out of a dozen samples of Aspirin tablets, 4 were found to exceed the B. P. specification for the limit of Salicylic Acid.

Samples of Salicylic Acid, all of wellknown make, failed to comply with only one B. P. requirement, namely, that of limit of iron and colouring matter. These samples in question were all of good quality and the particular B. P. test, we believe was too stringent.

Majority of the Calcium Gluconate solutions for parenteral use complied with the pharmacopoeial requirements, for the content of the active ingredients but some of them were found to contain precipitates or suspended

matter. This might have been due to the instability of the preparation or proper attention being not paid to the "clarity test" before the ampoules were packed for distribution.

Samples of Chloroform labelled as fit for anaesthetic purposes contained a decomposition product which made the use of such preparations unsafe.

Four samples of Acriflavin were tested during the year but all of them were of substandard quality.

A large number of edible and essential oils were examined out of which some of the samples of Olive Oil appeared to have been adulterated with Groundnut Oil. The latter was chosen as the adulterant due to its close similarity to Olive Oil in chemical and physical properties. Fortunately, such adulterants were readily detected by specific tests.

Some samples of Cod Liver Oil did not comply with the pharmacopoeial standard for acidity. In a few cases Code Liver Oil appeared to consist of Liquid Paraffin in which the content of Vitamin A had been carefully adjusted. Such oils have been found to be composed of almost entirely of unsaponifiable matter.

Several samples of Eucalyptus oil were examined according to Indian Pharmacopoeial List. Some of the samples did not comply with the minimum limit for cineole. It may be pointed out that in Indian Pharmacopoeial List the minimum limit for cineole in a sample of Eucalyptus oil is much lower than that prescribed in B. P.

It is noteworthy that almost all the samples of Emplastrum Belladonna examined in the department for the last few years showed rather poor alkaloidal content.

Examination of pepsin preparations indicated that such drugs have a limited stability if not properly prepared and stored.

Out of the eighteen samples of distilled water for parenteral use tested under the Drugs Act, five failed to comply with the requirements in respect of limits of oxidisable matter or ammonia or clarity.

About fifty per cent. of the samples of Absorbent Cotton failed to comply with the official requirements for absorbency and water soluble extractives. A large number of samples of Absorbent Gauze and Bandage did not comply with the test for absorbency or thread count.

About eighty per cent. of the samples of Absorbent Lint tested during the year failed to comply with standards for absorbency or superficial area per ounce.

The above results indicated that probably suitable standard raw cotton was not available with which cotton, gauze, bandage or lint for surgical use could be made to meet the official requirements. In fact, some of the specifications are now modified for Indian products.

Prohibition in the State has prompted certain pharmaceutical firms to put on the market obscure pharmaceutical tinctures of doubtful therapeutic efficacy. It has been the task of this laboratory to examine a number of

such preparations and the task was made more difficult due to the lack of adequate pharmacopoeial standards. On close examination some of these tinctures have been found to be nothing but coloured alcohol.

Examination of drugs by biochemical and microbiological methods.—Majority of samples tested by biochemical or microbiological methods were vitamins, namely, Thiamine, Riboflavin, Niacin, Calcium Pantothenate, Folic Acid, Ascorbic Acid, Methionine, B₁₂ etc. Samples of Insulin and enzymatic preparations were also analysed.

As many as 505 preparations containing vitamins were examined and amongst these, the so-called B-complex preparations, constituted a high percentage. These complex preparations, in some cases, contained in addition to common vitamins, different amino acids, extracts of liver, stomach, bone marrow, malt or yeast and sometimes enzymes and even in a few cases alkaloids. Owing to the complex nature of these preparations, the task of analysing these products often became very difficult and time-consuming. A critical analysis of these multivitamin preparations revealed that a good percentage of them failed to comply with the required standards, the reason being that one item or the other was found to be lower or higher than the stated amount. The proper understanding of the stability of various vitamins under different conditions and the incompatibility among the ingredients is essential before this class of preparations is put on the market.

Based on the investigation carried out here it has been repeatedly pointed out in our earlier reports that vitamin B₁ is unstable in certain oral multivitamin preparations. It was observed that in a number of such preparations Aneurine Hydrochloride was added in excess to counteract the loss of potency and this was indicated on the labels by some manufacturers. On the other hand, in some samples the amount of vitamin B₁ was found to have been added in excess although it was not mentioned on the labels of the containers.

Greater attention was paid during the year in developing microbiological techniques in evaluating the potency of some of the vitamins which could not otherwise be assayed by chemical means. The establishment of a satisfactory microbiological assay of vitamin B₁₂, one of the recently introduced vitamins, took considerable part of our time. Riboflavin, Nicotinic Acid and its amides and Calcium Pantothenate were tested microbiologically whenever required.

Recently it has been reported that the stability of vitamin B₁₂ is affected by its exposure to air. It has been observed here that in some oral pharmaceutical preparations, the potency of vitamin B₁₂ decreased on storage. One of the reasons for this might be due to its contact with the air.

Biological assay and Standardisation.—Rat uterus method was frequently used for assay of extracts of pituitary (post-lobe). This method which has been introduced recently is less time-consuming than the old classical method which required the use of guinea-pig's uterus. Of the five samples tested only one was found to be of substandard quality.

Forty-three samples of adrenaline were tested including fourteen under the Drugs Act. Out of these fourteen samples, ten showed potency lower than the prescribed limit. Cats were mainly used for these biological assays.

Both cats and guinea-pigs were used for the biological standardisation of Digitalis preparations. Out of the twenty-six samples of Tincture Digitalis assayed, five did not comply with the required standard. In two samples the glycosidal contents were very low.

Parenteral solutions numbering three hundred and eighty-two were examined for the absence of pyrogenic substances. The rabbits used for this test were kept at a constant temperature in an air-conditioned room specially built for the purpose. Only in a few samples pyrogenic reactions were encountered.

Some of the organo-metallic compounds, namely, Neoarsphenamine, Sulpharsphenamine, Antimonials, were tested both chemically and for the absence of undue toxicity.

Examination of drugs by bacteriological and serological methods.—Amongst the samples that were tested by the above methods, the following may be mentioned—antibiotics, surgical dressings, cat-guts, antitoxins, vaccines and injectables.

One hundred and forty-three samples of antibiotics (Penicillin G-Sodium 32, procaine Penicillin 32, Penicillin-Streptomycin 2, Other preparations of Penicillin 17, Dihydrostreptomycin 11, Streptomycin 2, Aureomycin 43, Chloromycetin 2, and Terramycin 2) were assayed. Along with the biological assay of Penicillin, chemical assay was also performed side by side in a good number of these samples. The results obtained by the two different methods showed remarkable agreement in most cases.

Sixteen samples of Procaine Penicillin, on examination, were found to be powdered tablets of sulphanilamide.

Out of the 43 samples of Aureomycin tested, 10 were found to contain a yellow powder which did not give tests for Aureomycin. Evidently these were spurious samples.

Nine samples of Tetanus Antitoxin and 5 samples of Diphtheria Antitoxin were tested. Potencies of these samples conformed to the claim made by the manufacturers.

Majority of the samples of Absorbent Gauze, Cotton and Bandage stated to be sterile did not comply with the requirement for sterility test. One of the reasons for their being non-sterile may be ascribed to the packing materials which might not have sufficient resistance to bacteria on storage.

Three hundred and sixty-four samples of injectables were tested for their sterility. Most of them were found to be free from bacterial contamination.

Research Work.—Summarized statements of the investigations carried out in the Department are given in Section II of this Report.

VIII. Department of Chemotherapy.

The regular manufacture of Sulphathiazole in the form of B. P. powder, tablets and injectibles was continued during the year, and the demands of the various Government and Municipal hospitals and public health bodies of Bombay as well as other States were completely met. Sulphathiazole was also made available to private and charitable hospitals and dispensaries and also to private medical practitioners. About 35,20,600 tablets, 363 lbs. B. P. powder and 19,500 ccs. injectible sulphathiazole were sold during the year.

The sales of Sulphathiazole in sufficient quantities and at comparatively low prices have, to some extent, influenced the foreign firms to bring down their current market prices which are now falling in line with our selling prices. Efforts were therefore made to bring down further the cost of manufacture of Sulphathiazole, in order to be able to make the drug available at still cheaper rates. Since the manufacture of Sulphathiazole was thoroughly standardised and the maximum possible yields in the various stages of the manufacture were being obtained, there was little possibility of an improvement in this direction. Further improvement in the matter of reduction of cost of production lies in two directions, viz. (1) to procure the raw materials without import duty or at concessional import duty, and (2) to bring down the expenditure incurred on the overheads. We pay approximately 37 per cent. as import duty on the raw materials imported from abroad, and reasonable reduction in this item, as has been done in the case of some other protected industries, would contribute towards material reduction in the cost of production. The second factor which would lead to comparative reduction of costs would be to increase the sales by making proper propaganda and advertisement for the products and thus indirectly reducing the overhead cost.

Investigations in connection with the manufacture of other sulpha drugs viz., Sulphamerazine, Sulphamethazine, and Sulphaguanidine are now in progress and are proceeding satisfactorily.

The stock of the antimalarial drug Proguanil Hydrochloride (Paludrine) which was manufactured from a number of experimental batches on pilot plant scale, was released to the Assam Government. Various other State governments also put in their indents for 'Paludrine', but the Department has not yet, however, undertaken the regular manufacture of this drug, pending instructions from the Government.

The demands for the antiseptic solution 'Pemon' were fully met with during the year under review. About 4,778 lbs. of Pemon were supplied. The department continues to prepare and supply phenyl mercuric nitrate which is being used as a preservative in the vaccines prepared in the Institute.

The preparation of Hydnocreol and Sulphetrone, the drugs used in the treatment of Leprosy was also continued. About 1,35,650 ccs. of Sulphetrone and about 330.5 lbs. of Hydnocreol were supplied to the Leprosy Clinics of the State.

Researches are being continued to develop newer drugs of potential value in the treatment of malarial and bacterial infections, and to determine their activities *in vitro* and *in vivo* tests.

The manufacturing processes as well as the various researches carried out on the theoretical and applied side demand a strict control and analysis. The analytical section of the department during the year under review carried out the complete control, analysis and testing of the various vitamin products and sulpha drugs manufactured. Besides, all the new organic compounds that were prepared in connection with the chemotherapy of experimental infections were analysed. About 700 such analyses were carried out.

Vitamin Tablet Section.—The manufacture and sale of the different kinds of vitamin tablets continued on an increasing level during the year under review. About 95,00,000 of vitamin tablets of different composition were sold during the year.

The question of the proposed sale of vitamin tablets through an approved agency is dropped for the present. A Business Manager has now been appointed and he is at present busy with reorganization of Stores and studying the general set-up and financial working of the Institute. As soon as this work is completed it is expected that he would take up the question of increasing the sales of various products manufactured in the Institute.

A comparative table is given below showing the sale of various kinds of tablets and their sale values during the periods 1951, 1952 and 1953.

A table showing the sale of vitamin tablets during the calendar years 1951, 1952 and 1953.

Serial No.	Kind of tablets.	Rate per 1,000 tablets.	Number of tablets sold during the period.		
			1951	1952	1953
		Rs.			
1	Vitamin A-D tablets	14	32,75,280	35,14,875	32,91,150
2	Vitamin A-D Special	20	99,000	88,000	1,32,000
3	Vitamin A Special	65	6,000	7,000	6,000
4	Vitamin B-C tablets	16	43,98,780	46,02,700	50,95,350
5	Vitamin B-C ₂ tablets	53	1,79,850	3,16,400	4,82,250
6	Vitamin B ₁ tablets	28	1,47,225	2,09,125	1,47,150
7	Vitamin B ₂ tablets	30	62,950	40,175	31,450
8	Vitamin C tablets	28	2,75,950	2,48,075	2,14,900
9	Acid Nicotinic tablets	6	1,31,030	1,36,650	97,700

Serial No.	Kind of tablets.	Rate per 1,000 tablets.	Number of tablets sold during the period.		
			1951	1952	1953
		Rs.			
10	Vitamin B-C Special ...	130	5,600	2,000	Nil.
11	Vitamin C Special ...	75	Nil.	Nil.	2,000
	Totalsale of vitamin tablets only.		85,81,665	92,55,000	94,99,950
12	Sulphathiazole tablets ...	35	27,13,525	19,42,500	35,20,600
	Totalsale of all kinds of tablets ...		1,12,95,190	1,11,97,500	1,30,20,550
		Rs. a. p.	Rs. a. p.	Rs. a. p.	
Total Sale-value of vitamin tablets only.			1,43,388 1 6	1,58,362 11 0	1,68,008 7 0
Total Sale-value of Sulphathiazole tablets only.			94,973 6 0	67,987 8 0	1,23,479 8 0
Total Sale-value of all kinds of tablets.			2,38,361 7 6	2,26,350 3 0	2,91,487 15 0

IX. Department of Biochemistry.

During the year under review clinical materials were received as usual from hospitals and private practitioners and the total number of analysis carried out increased to 7158. The following table gives a summary of the various examinations carried out during the year 1953 as compared against the previous three years :—

	1950	1951	1952	1953
Blood ...	1684	2170	2637	3752
Urine ...	194	179	149	280
C. S. F. and Body fluids ...	638	1236	1764	1324
Stool samples ...	6	3	9	14
Special tests ...	148	207	397	470
Milk samples ...	92	471	1596	1318
	2762	4266	6552	7158

The above figures clearly show that the pressure of work in the department has increased three fold since the year 1950, so much so that it has been impossible to expand the activities of the department and to devote any time to research problems of clinical interest. With the excellent facilities available in the department it is hoped to be of better service to the hospitals and to the public when more staff is made available.

As in the previous year the Seamen's Medical Examination Board referred some cases to us, 82 Seamen with glycosuric symptoms were examined for their glucose Tolerance. 1318 samples of milk were accepted for analysis during the year.

Research work.—Constant attempts are made to introduce up to date modifications and to develop simpler techniques particularly for quantitative blood analysis. For the estimation of potassium in serum a micro-method is under investigation—a method which would involve the least number of manipulations and be completed in a couple of hours.

The study of blood glycogen levels of normal as well as pathological cases has been taken up and is under progress.

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, and (5) conduct research in specific virus diseases like influenza, fever, rabies and small-pox.

1. *Anti-rabic Treatment Centre at this Institute.*—During the year under report 8714 persons applied for anti-rabic treatment; of those 7722 completed the full course of treatment; 985 were "advice cases," and 7 discontinued treatment. In cases where there was a doubt about the condition of the dog inflicting the bite and the dog was available for observation, 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 in Table I.

TABLE I—*Details of treatment carried out at the treatment centre of this Institute since 1950-53.*

Details	1950	1951	1952	1953
Number of persons who applied for treatment.	5,473	6,489	7,779	8,714
Advice cases	539	624	689	985
Fully treated	4,934	5,865	7,092	7,722
Discontinued treatment	Nil.	Nil.	Nil.	7
Reported cases of hydrophobia from those treated at the Institute Centre.	Nil.	Nil.	Nil.	1

Only one case developed hydrophobia in 1953 after prophylactic treatment. The patient, who had received 7 punctured wounds on the right arm, sought treatment 45 days later. In spite of Class III treatment given to him, he developed hydrophobia on the 12th day after receiving 12 injections of 10 cc. each. The failure of prophylactic treatment in this case was obviously due to severity of bite and the very long interval that elapsed between the bite and the commencement of treatment.

2. *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 145 to 153 during the year, out of which 67 were in Bombay State. The vaccine was also supplied to the Veterinary Surgeons within the State and outside for treatment of animals. 6,185 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 Carbolised Anti-rabic Vaccine and Canine Vaccine in ml. from 1949 to 1953.*

Details	1949	1950	1951	1952	1953
Anti-rabic ..					
Vaccine in ml. ..	16,55,920	16,72,840	19,40,215	23,39,740	26,10,160
Canine Vaccine in ml. ..	744	1,060	1,475	3,470	6,185

TABLE III.—*Production of Anti-rabic vaccine and supplies in ml. during 1953 month by month.*

Month.				Number of ml. vaccine manufactured.	Number of ml. vaccine issued.
January	...	...	...	1,91,860	2,13,033
February	...	...	...	2,67,500	1,81,451
March	...	...	...	2,13,740	2,48,698
April	...	...	...	2,48,200	2,04,259
May	...	...	...	2,56,420	2,02,600
June	...	...	...	2,68,580	1,87,127
July	...	...	...	1,94,660	1,88,237
August	...	...	...	1,51,240	1,45,450
September	...	...	...	1,65,280	1,44,647
October	...	...	...	1,72,720	1,88,334
November	...	...	...	2,15,520	1,83,875
December	...	...	...	2,64,440	2,37,256
Total	...	...	...	26,10,160	23,24,967

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.	1949	1950	1951	1952	1953
Number of treatments sent to all centres.	11,044	1,468	13,432	17,604	19,230
Number of treatments sent to Veterinary Surgeons.	675	606	646	827	804
Reported cases of hydrophobia.	Nil.	1	Nil.	6	3
Number of sheep used for production of anti-rabic vaccine.	957	1,040	1,342	1,337	1,545
Number of treatment Centres.	120	129	134	145	153

A total of 26,10,160 ml. of Anti-rabic vaccine was manufactured at this Institute and 23,24,967 ml. was supplied to various anti-rabic centres during the year. The total number of treatments issued during the year was 19,230 and when classified according to three classes showed the following distribution :—

Class I	...	1,130	treatments
Class II	...	9,708	treatments
Class III	...	8,392	treatments.
		<u>19,230</u>	treatments.

Eight additional centres for anti-rabic treatment were opened during the year taking the total number to 153. They are shown along with the dates of their opening.

Serial No.	Name of the Anti-rabic Centre.	Date of opening.
1	The Irwin Hospital, Jamnagar (Saurashtra)	.. 14th February 1953.
2	The N. M. Wadia Dispensary, Malegaon (Bombay)	... 25th February 1953.
3	The Hospital of Tata Chemicals Ltd., Mithapur (Saurashtra).	14th May 1953.
4	The M. R. Vila Hospital, Lathi (Saurashtra)	.. 19th May 1953.
5	The Shri Savar Kundla Vaidyakiya Rahat Mandal, Savar Kundla (Saurashtra).	23rd May 1953.
6	The Anand Municipal Dispensary, Anand (Bombay)	.. 22nd August 1953.
7	The Cottage Hospital, Dhanduka (Bombay)	.. 21st November 1953.
8	The Government Dispensary, Dhrol (Saurashtra)	.. 29th December 1953.

3. *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 materials is suitable only for 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 89 brain specimens received 47 were sent in glycerine and 42 in formalin. In addition to microscopic and histological examinations the number of biological tests carried out in this year in mice to diagnose rabies was 38.

TABLE V.—*Number of brain specimens received for diagnosis of rabies from Bombay City and out-stations during the year 1953.*

		Total	Positive	Negative	Unfit for examination.
Bombay City		42	20	21	1
Out-stations	...	47	18	24	5
Grand Total	..	89	38	45	6

TABLE VI.—*Statement showing the classification of different animals with the results of their brain examination for rabies during 1953.*

Animals.		Total	Positive	Negative	Unfit for examination.
Dog		74	36	36	2
Buffalo	.. .	4	...	2	2
Bullock		3		1	2
Cow		2		2	
Cat		2		2	
Goat		1	1		
Rabbit		1		1	
Human	.. ●	2	1	1	
Total	..	89	38	45	6

(4) *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, herpes simplex and rabies. These are reported separately under Part II.

This department also undertook an investigation of the mysterious disease from Nadiad, where water supply was thought to be infected; water samples received from Nadiad, were suitably treated and concentrated before they were inoculated into growing chicks embryos and laboratory animals as mice, guinea-pigs and hamsters but no virus could be isolated. A recent outbreak of infectious hepatitis localised to a Bombay Mill area was similarly investigated with negative results.

(5) *Yellow Fever Vaccine and Inoculation.*—The yellow fever diagnostic unit at the Haffkine Institute continued giving yellow fever inoculations to all passengers going to the yellow fever endemic areas, as well as it tested the potency of yellow fever vaccine stored here on behalf of Government of India. 13 potency tests were performed. The results so far show that the vaccine has retained potency for as long as five to seven years from the date of manufacture.

Table VII shows the number of persons inoculated against yellow fever during 1953. Figures for 1951 and 1952 have been given for purposes of comparison.

TABLE VII.

Month.				1951	1952	1953
January	..	..	..	667	753	598
February	..	..	..	705	570	818
March	..	..	..	529	462	916
April	..	..	..	883	719	976
May	..	..	..	3,250	779	1,204
June	..	..	..	571	859	846
July	..	..	..	250	685	1,011
August	..	..	..	567	706	752
September	..	..	..	595	663	635
October	..	..	..	667	785	759
November	..	..	..	694	650	693
December	..	..	..	817	722	891
				10,195	8,353	10,099

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

Centre.	Quantity of vaccine supplied in doses.	
	1952	1953
1. The Chief Medical Officer, Porbandar	480	280
2. The Port Health Officer, Jamnagar	400	500
3. The Director of Sanitary and Health Services, Ceylon, Colombo.	2,000	2,000
4. The Director-General of Medical and Health Services, Goa ..	560	900
5. The Provincial Public Health Laboratory, New Delhi ..	1,280	1,000
6. The King Institute, Guindy, Madras	2,000	Nil.
7. The All-India Institute of Hygiene and Public Health, Calcutta.	6,000	6,000
8. The Air Port Health Officer, Santa Cruz	760	1,040
Total ..	13,480	11,720

XI. Blood Bank Department.

During the year under report, the department continued its normal activities. The demand for dried plasma has shown a drop by about 30 per cent. This can be attributed to the introduction of synthetic plasma expanders like Dextran and P. V. P. which are now freely available in the market and the cost of these is much lower than that of dried plasma.

The demand for blood grouping sera has further increased and it was not possible to meet this demand by collecting sera from our normal donors. So some of the donors were immunised by injecting blood group substances and high titred sera prepared and issued.

As usual all blood samples were grouped and Kahn tests were performed in this department. The blood donors were screened for their fitness by determining haemoglobin level by Copper Sulphate Specific Gravity method.

The following is the account of the work done in the department during the year under report.

2,907 blood donors were screened by CuSO_4 specific gravity method for their fitness to give blood, out of which 1,586 donors were rejected giving a rejection rate of 54.5 per cent.

2,627 blood samples were tested for Kahn tests of which 478 samples were positive, i.e. 18.19 per cent.

16,200 ml. of blood with high titred haemagglutinin was supplied to the Diagnostic department for preparation of Blood grouping Sera.

2,404 samples of blood were grouped according to ABO system. The following is their percentage :—

AB—174 (7·2 per cent.)	.. B—772 (32·1 per cent.)
A—608 (25·2 per cent)	.. O—850 (35·3 per cent.)

Sixteen blood donors were immunised by injecting a saliva from secretors to enhance the titre haemagglutinins in their blood. Of these 6 were 'A' group donors, and 10 'B' group donors. 5,750 ml. of blood was collected from them for preparation of grouping sera.

TABLE NO. 1—*Showing the number of blood donors.*

Centre	Type of donors	No. of donors bled.	Total number of donors bled.
Haffkine Institute	... Paid	... 1,321	1,714
	... Voluntary	... 5	
	... Jail	... 388	
Indian Red Cross	... Paid	... 905	906
	... Voluntary	... 1	
Other Hospitals	... Voluntary	... 58	58
Grand Total		...	2,678

TABLE NO. II—*Showing the blood and plasma yield.*

Product	Quantity in litres	Bottles
Blood	... 684.600	
Liquid Plasma	... 388.250	
Dried Plasma	... 428.750	1,715

TABLE No. III—*Showing the distribution of plasma*

Nature		Quantity in litres	Bottles
Sold	...	222.750	891
Free	...	106.250	425
Total		329.000	1,316

The work on investigations of isoimmunisation due to Rh factor is continuing. The following table gives the work done in this connection.

No. of cases referred for investigation	..	..	275
Total number of individuals tested	..	..	446
No. of persons found Rh negative	..	..	35
Per cent. of Rh negative persons	..	..	7.8

Sera of 2 isoimmunised mothers was found suitable for use as Anti-Rh grouping sera and was collected for that purpose.

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 1953 :—

Serial No.	Name.	Designation and address.	Department in which trained.	Duration of training.
1	Smt. A. N. Gajtonde, L.C.P. & S.	Medical Officer, Municipal Dispensary, Malegaon.	Virus Diseases including Rabies	7th March 1953 to 14th March 1953.
2	Dr. Rose M. Smith ...	Medical Officer, W. F. Pierce Memorial Hospital, Wai.	Do. ...	15th April 1953 to 21st April 1953.
3	Dr. J. J. Parikh ...	Medical Officer, I/c. Shree Savar Kundla Vaidya Rahat Mandal Savarkundla.	Do. ...	13th May 1953 to 19th May 1953.

Serial No.	Name.	Designation and address.	Department in which trained.	Duration of training.
4	Dr. Smt. Goradia ...	Medical Officer, Tata Chemicals Ltd., Mithapur.,	Virus Disease including Rabies.	11th June 1953 to 17th June 1953.
5	Dr. Makhanlal Jain, M.B.	Private Medical practitioner, Sagar.	Clinical Pathology	2nd March 1953 to 31st March 1953.
6	Shri M. M. Deshpande, M.Sc.	56, N. C. Kelkar Road, Dadar, Bombay.	Do. ...	17th September 1953 to 30th November 1953.
7	Dr. Shamlal Kaul ...	Anesthetist, S.M.H. S. Hospital, Jammu and Shrinagar.	Blood Bank ...	31st October 1953 to 30th November 1953.
8	Dr. Vasudev ...	Anesthetist ...	Do. ...	Do.
9	Dr. S. Upadhyaya ...	Medical Officer, Plague Laboratory, Hyderabad (Deccan).	Vaccine ...	23rd February 1953 to 15th May 1953.
10	Shri C. J. Mody, B.Sc.	Chemist, M/s Alem-bic Chemical Works Ltd., Baroda.	Animal House ...	1st April 1953 to 30th May 1953.
11	Shri Y. G. Bhide B.M.S., Cl. II.	Blood Transfusion Officer, J. J. Group of Hospitals, Bombay.	Blood Bank ...	5th October 1953 to 24th October 1953.
12	Dr. Minguel Antonio J.E.P. Alfonso.	Institute Bacteriologico, Goa.	Clinical Pathology	16th March 1953 to 11th July 1953.
13	Dr. S. S. Suvi ...	Assistant Director, Harcourt Butler Institute, Rangoon.	Vaccine and Antitoxins and Sera. ...	14th September 1953 to 21st September 1953.

XIII. Critics Club.

Under the auspices of this Club, 44 Weekly Scientific Meetings were held during the year. Abstracts of current scientific literature or talks were given during these meetings by members of the various Departments of the Institute. A number of special talks were also arranged. These were as follows :—

Speaker.	Subject
(1) Sir Alexander Fleming	.. The Story of Penicillin.
(2) Dr. Corneille Heymans	.. Survival and Revival of Nerve Centres after the onset of Circulation.
(3) Dr. J. N. Vajifdar	.. Blood Banks in the U. S. A.

XIV. Miscellaneous.

Apart from the researches carried out by the members of the staff, the following scientific enquiries sponsored by Indian Council of Medical Research were also conducted during the year 1953.

1. Plague Researches .. Under Dr. P. M. Wagle, Director,
Haffkine Institute, Bombay.
2. Enquiry on the Role of Nutri- Under Dr. M. V. Radhakrishna Rao,
tional Factors in Hepatic Assistant Director, I/c Depart-
Cirrhosis : ment of Nutrition.
3. Enquiry on the Study of Indian Under Dr. P. J. Deoras, Assistant
Poisonous Snakes. Director I/c Department of
Entomology.

In addition to above-mentioned scientific enquiries conducted here, the following research fellowships and scholarships were attached to this Institute during the year 1953. Particulars of the sponsors, the subject of fellowship and the candidates working under them are as under:—

Serial No.	Sponsors.	Subject of Fellowship.	Candidate working on the Fellowship.
1	Unichem Laboratories, Bombay.	Researches on Casein Hydrolysate.	Dr. K. C. Thadani, B.Sc., Ph.D.
2	Do. do. ...	Researches on Indigenous Drugs.	Shri B. 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.
4	Tata Industries Ltd., Dept. of Industrial Health, Bombay.	Entomological Survey of Kurla and its environments.	Shri D. R. Ranade, M.Sc.

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., and (2) Shri M. S. Gokhale, M.Sc.

Lastly, I may also mention of the facilities offered to science graduates for doing their post-graduate degrees by thesis at this Institute. During the year 1953 the following candidates were given such facilities under this scheme—

- (1) Kumari R. S. Vakil, B.Sc. (completed 30th September 1953).
- (2) Shri K. V. Tonpi, B.Sc.
- (3) Shri P. Y. Dighe, B.Sc.
- (4) Shri N. V. Bhagvan, B.Sc.
- (5) Shri K. V. Pai, B.Sc., (discontinued from 24th August 1953).
- (6) Shri S. V. Nabar, M.Sc. (discontinued from 28th June 1953).
- (7) Shri S. M. Vaidya, B.Sc.

XV. Income and Expenditure 1952-53.

Receipts.	Expenditure.
XXVII— <i>Medical Bacteriological Laboratories—</i>	38— <i>Medical—</i>
Rs.	
Sale proceeds of Sera and Vaccines. 17,98,150	<i>H. Bacteriological Laboratories—</i>
Other Miscellaneous Receipts. 58,226	<i>H. 1. Haffkine Institute—</i>
	Rs.
	Pay of Officers ... 2,03,546
	Pay of Establishment ... 4,16,407
	Allowances etc. ... 3,27,850
	Contingencies ... 11,37,097
	Research Scholarships ... 2,424
	<i>H. 2. Pasteur Institute—</i>
	Pay of Officers ... 11,165
	Allowances etc. ... 3,153
Total Income ... 18,56,376	Total expenditure ... 21,01,642

PART II.

RESEARCH WORK.

I. Nutritional Requirements of *P. Pestis*.

(WAGLE AND GOVIND).

In the previous reports it has been stated that the influence of nuchar in enhancing the growth of *P. Pestis* is neither due to (1) any amino-acid imbalance in the casein hydrolysate nor to (2) removal of any inhibitory factor from the casein hydrolysate. It was then considered that charcoal might act as a reducing agent in the medium. In the last year's report evidence has been adduced to show that growth of small inocula of *P. Pestis* on nutrient agar is considerably enhanced by the addition of one per cent. charcoal to the agar.

In order to determine the reducing effect of charcoal the following experiment was undertaken. Two isonitrogenous sets of casein hydrolysates, one charcoal treated and the other without charcoal treatment were prepared from the same batch of casein, and inoculated with *P. Pestis* (same inoculum 0.05 cc. of 24-hour casein hydrolysate culture grown at room temperature). The potential of the medium was tested with the usual Eh (oxidation-reduction indicators, such as benzyl viologen, neutral red, resinduline, phenosafranine Janus green, methylene blue, tolylene blue, o-cresol indeophenol and phenol blue. Changes in the oxidation-reduction potential are indicated by changes in the colours of these indicators. There were no observable differences in the colours produced in the two media during growth up to 15 days. This showed that charcoal by itself did not effect any changes in the redox potential of the media.

In order to see whether charcoal treatment in any way affects the reducing activities of *P. pestis* tetrazolium bromide (trade name Grodex May and Baker product), which is unaffected by interfering substances, was used to test the reducing activities of *P. pestis*.

Triphenyl tetrazolium bromide is soluble in water and forms a colourless solution but in its reduced state (triphenyl formazon) the indicator assumes an intense red colour and is also insoluble in water. Triphenyl formazon is extracted by ether and since this indicator is unaffected by other reducing substances, any appreciable reduction of TTB to formazon may be ascribed to the reducing systems of bacteria during their growth and metabolic activities.

The colour intensities were compared in a Gallen Kamp photoelectric colorimeter using Illford filter No. (22). A straight line was obtained for formazon concentration ranging from 0 to 20 r/ml. Triphenyl formazon for standardization was prepared from TTB. by treatment with an equivalent quantity of ascorbic acid in presence of alkali.

0.5 cc. of a one per cent. solution of Grodex was added to 10 cc. of two sets of tubes of casein hydrolysates, one treated with charcoal and the other not so treated. It was observed that the reduction of TTB to formazon in

plague broth (treated with charcoal) was about 5 to 7 times that in casein hydrolysate not treated with charcoal, although the charcoal-treated gave only twice as much growth of *P. pestis* as the untreated broth. This evidence suggests that charcoal treatment of the broth considerably enhances the reducing systems of bacteria during their growth and metabolic activities.

At the suggestion of Sir Alexander Flemming that charcoal might probably remove certain inhibiting fatty acids which may be present in commercial casein, the influence of addition of graded amounts of oleic acid on the growth of *P. pestis* in casein hydrolysate was investigated. Five hundred mg. of oleic acid originally dissolved in ethyl alcohol was later on diluted with water so as to contain $0.5 \times 10_5$ to $0.5 \times 10_7$ of oleic acid in casein hydrolysate broth. In the concentrations tested, oleic acid did not show any demonstrable effect on the growth of *P. pestis*.

II. Passive Immunity Tests with Anti-Cholera Sera.

(WAGLE AND HABBU)

The test was employed to assay the comparative mouse protective values of the casein hydrolysate cholera vaccine and the agar-grown cholera vaccine.

Two sets of 4 rabbits were immunized with 3 doses of the two vaccines, viz., 0.5 cc., 1.0 cc. and 1.0 cc. given intravenously at weekly intervals. The animals were bled a week after the 3rd vaccine dose and the pooled sera assayed for mouse protective antibodies.

For assaying the sera five batches of 6 white mice (18-20 gm.) each were given graded doses of the sera, as shown in the table, so that the selected doses were contained in 0.5 cc. of the diluent. The serum dilutions were given intraperitoneally. Six hours later, the animals were given the challenge dose of about 1,00,000 mucinised vibrios intra-peritoneally. Two batches of animals were immunised with the same serum. One batch was challenged with Inaba test infective dose and the other with the Ogawa test infective dose to find out the protective power of the serum and consequently of the antigenic value of the vaccines against the two subtypes of *V. cholerae*.

With the results thus obtained the 50 per cent. end point was calculated by the method of Reed and Muench.

The results of two vaccines showed no significant differences. They are given in the attached table.

Passive Immunity Tests in Mice with Anti-Cholera Sera.

Experiment No.	Serum prepared with	Challenge strain.	Dose of Serum in cc.					Controls	Mouse Protective Dose.
			1.25×10^{-5}	2.5×10^{-5}	5.0×10^{-5}	10×10^{-5}	20×10^{-5}		
1	(a) Casein hydrolysate vaccine ...	Inaba 5597.	4/6	4/6	3/6	1/6	0/6	6/6	3.6×10^{-5} cc.
	(b) Agar Vaccine ...	...	5/6	3/6	2/6	1/6	1/6		3.4×10^{-5} cc.
2	(a) Casein hydrolysate vaccine ...	Inaba 5597.	5/6	5/6	1/6	2/6	1/6	6/6	4×10^{-5} cc.
	(b) Agar Vaccine ...	...	6/6	4/6	3/6	2/6	1/6		5.6×10^{-5} cc.
3	(a) Casein Hydrolysate Vaccine.	Ogawa 1A.	6/6	3/6	1/6	0/6	0/6	6/6	2.8×10^{-5} cc.
	(b) Agar Vaccine ...	...	6/6	1/6	1/6	1/6	1/6		2.3×10^{-5} cc.
4	(a) Casein hydrolysate Vaccine.	Ogawa 1A	5/6	6/6	5/6	1/6	2/6	6/6	7.5×10^{-5} cc.
	(b) Agar Vaccine ...	...	5/6	5/6	3/6	6/6	1/6		11.0×10^{-5} cc.

III. Bactericidal Titre of Anti-Cholera Sera as a Measure of the Antigenic Value of Cholera Vaccine.

(WAGLE AND HABBU)

Ahuja, on the basis of his experiments, reported that rough or smooth phase of cholera vibrios could be easily ascertained by incubating peptone water cultures of these organisms with fresh g. pig complement at 37°C for 4 hours. He found fresh g. pig complement was highly bactericidal to rough strains whereas smooth strains actually grew in it. Ahuja went a step further and advocated that the antigenic value of cholera vaccines, prepared from smooth immunogenic strains which can grow in the presence of fresh g. pig complement and from rough non immunogenic strains which are killed by fresh g. pig complement, could be measured by obtaining immune sera from these vaccines and measuring their bactericidal titre which is a direct measure of the antigenic value of these vaccines.

For testing the validity of this hypothesis two groups of 4 rabbits were immunised with two different cholera vaccines. Three doses of 0.5 cc., 1 cc. and 1 cc. of the vaccines were given to the rabbits at weekly intervals, and rabbits were bled a week after the 3rd vaccine dose. Bactericidal tests were put up by the technique of Ahuja *et al* (1948). It was observed that the bactericidal titre of the immune sera fell down rapidly from original titre of 1:100,000 to 1:100 at the end of 3 weeks though the sera were well preserved. During the same period the mouse protective values of the sera did not change. It is, therefore, evident that the bactericidal titre bears no correlation whatsoever with mouse protective titre of the serum and would, therefore be unsuitable for the measurement of the antigenic value of the cholera vaccine unless the tests are carried out immediately after the preparation of the sera.

IV. 44°C. Mackonkey Test for Identification of B. Coli in water Analysis.

(NIBKAR, RAJADHYAKSHA AND HAZRA).

44°C Mackonkey test as modified by Wilson is recommended for distinguishing B. Coli Type I (faecal) and other coliform organisms of I. A. C. group. It is based on the fact that B. Coli type I (faecal) multiply and produce gas in Mackonkey broth when incubated at 44°C while organisms of the I. A. C. group fail to do so. This test has been adopted by many laboratories for rapid identification of B. Coli.

However, in our country it has been observed by some workers in Madras and Bengal that about 5-10 per cent Aerogenes like organisms isolated from water and faeces gave a positive 44°C Mackonkey test.

With a view to study the possibility of the application of this reaction for identification of coli form organisms isolated from this part of the country this work was undertaken.

The coliform organisms isolated during the routine bacteriological examination of water and food samples by plating method have been subjected to confirmatory tests such as Methyl red, Voges proskavver test, Indole production and Koser's citrate utilisation test as well as to the 44°C Mackonkey test. The results of the latter were compared with those of the other tests mentioned above.

44°C Mackonkey test is carried out in an accurately controlled waterbath fitted with an electric stirrer. The bath maintains a temperature of 44°C with a variation of 0.2°C to 0.5°C. Gas production was observed at 18, 24 and 48 hour intervals. So far only 13 strains, mainly isolated from water, have been studied. Out of these six showed typical reactions of *B. coli* type I (faecal) and all gave a positive 44°C Mackonkey test within 18 hours. Three strains were classified as *Aerogenes* type I by confirmatory tests and all these strains gave a negative 44°C test up to 24 hours. However, one of these three strains showed gas production within 48 hours while the other two remained negative throughout that period.

One strain classified as *Aerogenes* type II, one as intermediate type I, one as intermediate type II and one irregular tests were all negative to 44°C Mackonkey test up to 48 hours. Further work is in progress.

V. Effect of Bentonite (Montmorillonite) on the Antibodies of Concentrated Immune Horse serum Globulins.

(MORE AND RAO).

Preliminary experiments indicated that when anti-toxic globulin was treated with bentonite solution the anti-toxic titre increased without loss of total antibody content. A purer antibody was thus obtained.

Bentonite treatment was carried out briefly in the following way:—

A stable suspension of 3 per cent. bentonite was prepared in distilled water and added to concentrated globulin solution diluted with equal part of physiological saline. pH of the mixture was adjusted to 5.5, shaken gently and allowed to stand at room temperature for half an hour and centrifuged at 2,500 RPM. The clear supernatant fluid was further treated likewise 3 times more and finally the anti toxic globulins were concentrated by precipitation with ammonium sulphate and dialysis in distilled water.

Anti toxic titre of the dialysed solution was determined by Ramon's Flocculation test. In case of 6 experimental lots the titre was found to have increased by 17 to 41 per cent. per mg. of protein nitrogen. The rise in anti-toxic titre may be due to removal of lipo-proteins which are non-anti-toxic, by adsorption on bentonite. Further work on lipid and electrophoretic analysis of bentonite treated globulins is in progress.

VI. Common Antigens in the venoms of Indian poisonous snakes viz. Cobra, Krait, Russell's Viper and Echis carinata.

(KULKARNI AND RAO).

Results of homogeneity of snake venom antigens by semi-solid precipitin technique revealed that each venom consists of several antigens. Electrophoretic analysis of these venoms at different pH also supported these findings. It was of great interest to know if venoms of four different snakes, viz. Cobra, Krait, Russell's viper and Echis had any common proteins. This study would also throw light on the genetic relationship of these snakes to one another.

Oudin's technique modified by Bowen was used for these studies. Monovalent horse sera and monovalent rabbit sera prepared by immunizing the animals with individual venoms were used. Solutions of lyophilised standard venoms of the above mentioned snakes diluted to required strength were used as antigen solutions.

For finding the common antigens between venoms, monovalent antisera prepared against one kind of venom was put against the venom of another kind. Observations were taken when no more bands appeared in the middle gel layer.

The number of precipitation bands in heterologous series represented the minimum number of common antigens. Following were the results obtained.

1. Cobra venom was found to have 8 antigens and Krait venom five antigens in homologous reactions. In cross reactions Cobra venom and Krait venom showed at least two antigens in common, one being present in large amounts and the other in smaller amounts.

2. Russell's Viper Venom was found to contain 9 antigens and Echis venom 7 antigens of which 4 were in common with Russell's viper venom according to cross reaction results, one of which is present in large amounts and other three in small amounts.

3. Cobra Venom and Russell's viper venom have at least 1 antigen in common present in medium strength.

4. No antigens were found common in Krait venom and Russell's viper venom or Krait venom and Echis venom.

Conclusion.—Venoms of the same species could be expected to have antigens in common. Cobra and Krait both belong to elapidae species and have at least two antigens in common; similarly Russell's viper and Echis which belong to viperinae species, have four antigens in common.

Venom of Cobra which belongs to elapidae having common antigen with venom of Russell's viper which belongs to viperinae species indicates that probably Cobra is an interlinking type between Elapidae and Viperine species of snakes.

VII. Action of Crystalline Trypsin on Crystalline Egg Albumin.

(SHRIMATI SHANTA RAO)

After overcoming a number of difficulties, crystalline trypsin and crystalline egg albumin were prepared successfully.

Crystalline trypsin did not act on crystalline egg albumin. However, heat denatured egg albumin was readily digested by crystalline trypsin.

Crystalline egg albumin treated with 0.3 per cent. or 0.6 per cent. formalin was resistant to the action of trypsin before or after heating at 100°C for 30 minutes. Formalinized egg albumin did not coagulate even on heating at 100°C for 30 minutes.

Amino acids released during the action of crystalline trypsin on heat denatured egg albumin was followed by paper chromatography. Arginine was the first amino acid to be released followed by lysine. Later, glutamic acid, tyrosine, tryptophane and leucine were detected. In the 32 hours digest, histidine, glycine, threonine or alanine, methionine and serine were also detected.

VIII. Fractionation of the soluble antigens of plague.

(BHAGAVAN AND RAO)

Ammonium sulphate fractionation method was used in fractionating the antigens of plague. Five fractions were obtained, precipitating at 30 per cent. (I), 35 per cent. (II), 40 per cent. (III), 50 per cent. (IV), and above 50 per cent. (V) concentration. More than 50 percent. of the total solids precipitated at 0-30 per cent. ammonium sulphate concentration. It was found that none of the fractions were homogeneous. Fraction I contained at least four antigens, out of which one was present in large proportion. The fraction II contained at least three antigens, and the remaining fraction contained more than three antigens each.

More encouraging results have been obtained by extraction of antigens with solvents like ethylene glycol and Diethylene glycol and fractionating with acetone.

IX. Optimum time required for action of venom at different levels in cobra venom

(KULKARNI AND HAZRA)

Cobra venom sample from large pool of lyophilised venom was used for these experiments.

Venom was diluted to give different strengths and 0.5 cc. of each dilution was administered intravenously into mice weighing 20 grms. each. For each dilution 20 mice were used. The time of injection of venom dose and time of death was carefully noted and mean time required for death for each dilution was calculated.

It was generally observed that time of death varied inversely with dose of venom injected to mice. The values of logarithm of time required for death when plotted against logarithm of quantity of venom injected gave a straight line.

On the lines of Christensen and Phinney equation $\log T = (\log K + \log d + \log t) - \infty \log D$, was applied and K for the sample of venom was found. Making use of the standard curve obtained with pooled sample, it is possible to standardize any Cobra venom.

Value was the slope of straight line obtained by plotting $\log T$ and $\log D$.

X. Survival time as affected by the interval between the Administration of Venom and Antivenine.

(HAZRA AND KULKARNI).

In the first series of experiment, Bgroups of mice of 20 gm. each were injected intravenously with varying dosage of Cobra Venom, viz. 2, 4 and 6 c. l. ds. This was followed 5 minutes later by intravenous injection of Antivenine in dosage capable of neutralizing 15 c. l. ds. of Cobra Venom.

The administration of antivenine 5 minutes after the venom dose did not afford complete protection against 2, 4 or 6 cl. ds. But, as compared to controls it was noted that survival time in the treated animals was prolonged. Secondly, the survival time in cases of treated animals was found to be decreasing with the increase of venom c. l. ds.

In the second series, as in the first, groups of mice were injected a constant 2 c. l. ds. of Cobra Venom.

This was followed at intervals of 10, 15 and 20 minutes by varying doeses of Antivenine capable of neutralizing Cobra Venom from 3 to 16 c. l. ds.

In this series too, the administration of Antivenine in varying doses after 10 and more minutes of venom injection failed to protect mice against as low as 2 c.l ds. of venom. But, as in the first antivenine constant series, the survival time of the treated animals was prolonged as compared to the controls in this series, with varying doses of Antivenine. In the differnt groups of this series with varying amount of Antivenine, it was noted that with Antivenine diluted to neutralize 10 or less c.l.ds. protected the mice against a constant 2 c.l.ds. of Cobra venom larger than antivenine in longer dilution to neutralize 10 and higher c.l.ds.

This may be due to the fact that dilution of Antibodies helps neutralization of Antigen better.

From the above experiments, it can be deducted that *in vivo* the neutralization of venom varies inversely with the time interval between venom and antivenine administration. This was not appreciably influenced by increasing the dose of antivenine injected. Further studies are in progress.

XI. Test Toxin and Standardization of Anti-Tetanus Serum.

(HAZRA AND MORE).

The role of 'test toxins' influencing the antitoxic titre obtained for a particular Tetanus Antitoxic Serum has been observed and reported in the past. The exact part played by a Test Toxin has been variously studied, but so far no definite acceptable conclusions are arrived at.

In our routine course of Tetanus Antitoxic sera standardization, we had occasion to observe different values for the same serum when tested against test toxins of varying age. Amongst other factors responsible for such results it was possible to study the influence of age of the Test Toxin, since some old test toxin was made available through the courtesy of a commercial firm. Thus a batch of sera prepared at the Institute was tested for its potency against Test Toxins of different ages.

One of the toxins (Lot 13) used for the test was prepared freshly by growing CT3 strain of *clostridium tetani* in veal infusion broth, for 48 hours and the toxin obtained after seitz filtration. The other toxin (lot No. 447B) was also prepared similarly but it was 8 years old, kept in sealed ampoules at 0°C. The L+ doses of the two toxins as tested subcutaneously in mice and guineapigs at one tenth International Unit level were found to be 0.11 ml. and 0.04 ml. respectively.

The L+ dose of the 8 year old toxin had remained the same, viz. 0.04 ml. for the period of storage, which in some other cases had been observed to vary with the age of the toxin.

Certain lethal doses of the two toxins tested on mice were found to be 1×10^4 for lot No. 447B and 3.3×10^{-5} for lot No. 13. This showed that the old toxin was less toxic but had a greater binding power than the freshly prepared one.

On testing the Tetanus Antitoxin (V 1262) against Test Toxin lot 13 the value obtained was 1000 I. U. per cc., whereas the value obtained against Test Toxin lot 447B was 2000 I. U. per cc.

The old toxin gave the Antitoxin a unit content value almost cent per cent. higher than the value given by the relatively fresh toxin.

The difference in value was repeatable and on repeated tests the average values obtained confirmed the above observation.

Similar observations, obtaining a higher potency value for a given serum with older test toxins as compared to values obtained against relatively recent toxins were observed with other batches of sera and Test Toxins. The above-mentioned observations raise the question of avidity, as well as dissociation of Toxin-Antitoxin mixtures as a cause of variation in the titre obtained.

Further work is in progress and the problem will be studied for effects of aging and other chemicals on the test toxins.

XII. Pulicidal value of pyrethrum.

(DEORAS AND GODBOLE).

Ninety one preliminary experiments done during the year have shown that the powder formulation of Pyrethrum extract mixed with the booster Piperonyl-butoxide gave the residual pulicidal effect upto 336 days when tested in glass jars. This residual effect compares favourably with DDT in the same formulations. Powder formulations of Pyrethrum with a booster gives better residual effect than solution and emulsion formulations.

XIII. Entomological Survey of the Swadeshi Mills, Kurla.

(DEORAS AND RANADE).

During 1953, different adult insects were collected each day by hand net in this mill area. Larvae found breeding in accumulated waters in different sectors were also collected and bred in the laboratory to adult stage. An account was kept of different ailments for which the workers attended the outpatient dispensary in the mill area each month. At the end of 12 months, 28127 adult insects belonging to 50 families of different orders had been collected. Of these, 14 families belonging to the Order Diptera may be of medical importance. Table No. IA and IB show some of the insects of probable medical importance collected and identified by the British Museum (Natural History), London, and a list of few ailments for which the workers attended the mill dispensary.

TABLE No. IA.

Showing insects of medical importance collected in the Swadeshi Mills Area, Kurla.

	January	February	March	April	May	June	July	August	September	October	November	December	
1. Anophelinae	...	...	12	11	8	2	...	6	10	11	7	14	15
2. Borboridae	...	...	3	...	...	...	...	...	...	...	...	...	...
3. Calliphoridae	...	...	3	2	...	...	...	6	15	7	13	34	2
4. Ceratopogonidae	...	...	35	10	8	10	14	7	15	20	29	29	37
5. Chironomidae	...	38	598	3,317	6,231	8,497	315	128	222	408	190	35	47
6. Chloropidae	...	10	12	...	8	8	5	...	14	...	32	...	3
7. Culicinae	...	...	20	12	6	...	...	67	21	13	13	16	15
8. Diopsidae	...	...	1	...	...	...	...	22	...	...	...	1	...
9. Dolichopodidae	...	...	...	3	...	...	...	...	...	...	...	2	...
10. Ephydriidae	...	6	220	140	80	187	46	674	317	358	118	226	226
11. Ottidae	...	...	...	...	...	...	...	...	...	2	2	8	...
12. Psychodidae	...	...	15	16	2	...	...	3	16	13	...	11	32
13. Muscidae	...	21	76	28	25	82	41	207	228	308	198	183	132
14. Sarcophagidae	...	...	1	...	...	...	...	1	...	...	1	...	...
15. Sepsidae	...	...	6	14	2	17	...	16	...	...	20	129	113
16. Stomoxydinae	...	...	...	1	1	...	4	4	3	13	8	6	...
17. Tipulidae	...	...	...	...	...	...	...	...	...	40	6	...	...

TABLE No. IB.

Showing cases treated in the Swadeshi Mills Dispensary during January-December 1953.

Disease	January	February	March	April	May	June	July	August	September	October	November	December
Malaria	... 44	42	31	37	34	24	33	46	45	33	26	27
Dysentery	... 15	21	33	18	14	24	53	73	65	45	20	15
Other gastric troubles	242	163	212	149	206	162	162	278	254	265	145	121
Filariasis		...	...	1	2	...	...	...	...	...	1	...
Helminthiasis		...	...	50	55	51	51	64	62	58	32	49
Guinea worms	... 1	1	2	7	8	4	3	2	...	2	...	2
Trachoma	... 2	1	2	...	...	1	1	3	1	1	1	4
Eye d'seases	... 22	17	18	24	16	32	19	22	17	34	18	21
Scabies	... 42	31	43	43	57	50	76	54	56	40	41	30
Respiratory Diseases	... 235	162	197	185	242	208	176	250	255	244	221	194

XIV. Some observations on snake blood.

(DEORAS AND MHASALKAR).

The table No. II gives results of the clinico-pathological and biochemical studies of blood of some poisonous snakes as compared to rat, rabbit and a frog. These observations may be useful in the future phylogenetical studies on reptiles in general and Ophidia in particular.

TABLE No. II.

Results of clinico-pathological and biochemical examination of some Mammal and Reptile Bloods.

No.	ANIMAL	CALCIUM mgms. per 100 cc. serum.	MAGNESIUM mgms. per 100 cc. serum.	INORGANIC PHOSPHOROUS mgms. per 100 cc. sera.	PHOSPHATASE ACTIVITY (BODANSKY UNITS)	ERYTHROCYTES millions per cu. mny. blood.	LEUCOCYTES per cu. mm. blood.	HAEMO- GLOBIN gms. per 100 cc. blood.
1	Rat ...	11.1	2.66	5.40	2.93	6.12	14,300	13.95
2	Rabbit	12.9	3.9	5.19	3.06	6.19	8,800	15.25
3	Frog ...	11.6	3.40	5.25	1.96	0.69	18,300	7.31
4	Lizard	1.2	3.95	6.70	2.20	0.62	10,650	6.80
5	Ratsnake	17.4	3.24	6.57	1.40	0.97	14,000	7.63
6	Cobra	18.0	5.12	5.38	2.50	1.49	9,447	6.20
7	Krait ...	19.2	3.50	5.60	1.64	1.34	22,350	7.73
8	Russell's Viper ...	18.5	4.56	6.51	1.00	1.46	13,333	6.88
9	Echis	18.4	4.20	5.02	1.36	1.44	26,000	7.08

XV. Sex Ratio in Flea Collections.

(DEORAS AND TONPI)

Collection of fleas from *Rattus rattus* and *G. kok* brought to the laboratory in uncovered cages has shown some interesting results.

Total number of rats examined were 815, out of which 461 were *Rattus rattus* and 354 *G. kok*. The number of *X. cheopis* recovered from *Rattus rattus* were 666 and *X. astia* 82; while in *G. kok* these numbers were 318 and 223 respectively. The sex ratio of male to female of *X. cheopis* on *R. rattus* and *G. kok* both, comes to 1:0.75 while that of *X. astia* in these rats was 1:1.41 and 1:2.66 respectively. The total number of fleas recovered from both the rats were 1,289. Of these 984 were *X. cheopis* and 305 *X. astia*. The sex ratio of male to female in this collection was 1:0.75 in *X. cheopis* and 1:2.32 in *X. astia*.

These studies are being continued to elucidate if there is any significance in the sex-ratio of the fleas. The life-history of *X. cheopis* done in the laboratory has shown that it takes 41 days to complete the cycle in December while only twenty days in June and July.

XVI. Chloromycetin in Typhoid Fever.

(DOGRA AND MODI)

Investigation in connection with effects of Chloromycetin in culturally positive enteric group of fever cases was continued at Sir Hurkisondas Hospital as in the previous year. Clinical records were obtained. Repeated blood cultures and triple Widal tests were made. Urine and stools were cultured at regular intervals. Another sixty cases were investigated during the period under review.

Follow up of convalescent cases continued to be unsatisfactory as none of the cases co-operated in submitting stool and urine samples after discharge from the hospital despite efforts to collect samples from patients' home.

Amongst this group of cases, Synthomycetin and Kemycetin were used in certain cases, instead of Chloromycetin, as these drugs were cheaper in price. Clinically, these drugs were almost similar to Chloromycetin.

Of the 97 cases studied so far, it may be concluded that (a) the blood culture becomes negative in majority of cases in 3-4 days after antibiotic therapy, but, it may become positive during a relapse; (b) the triple Widal test showed no rise in titre in majority of cases after treatment with Chloromycetin; (c) the incidence of relapse, complications and mortality rate showed no marked difference in variously treated groups.

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. 691 samples of urine were tested for presence of gonadotropic hormone. Including the previous

year's samples, a total of 1,214 samples have been examined. Of these, so far, 250 follow up cards have been returned completed. Analysis of these cards showed the following results:—

1. *The Positive Cases.*

			By Rat Test (90)	By Rabbit Test (25)
(1) Pregnancy	...	...	38	18
(2) Ectopic	...	...	5	3
(3) Abortion or miscarriage	...	...	19	2
(4) Moles	...	...	25	2
(5) Pregnancy with tumour	...	...	3	0

2. *The Negative Cases.*

			By Rat Test (85)	By Rabbit Test (50)
(1) Functional Amenorrhoea	...	...	54	32
(2) Lactation Amenorrhoea	...	...	1	0
(3) Menopausal	...	...	3	2
(4) Post abortal or missed abortion	...	...	11	8
(5) Pseudopysyesis	...	...	1	2
(6) Tumours and Pelvic Inflammation	...	...	9	5
(7) Mole after evacuation	...	...	6	1

It will be observed that in all these cases the rat and the rabbit tests showed complete agreement with the clinical findings. Further reports are expected when they will be analysed to ascertain the percentage agreement in a larger series of cases.

Whereas the rabbit and the rat tests were quite satisfactory in our hands they both required observation of animals for 24 to 48 hours—a period rather long from the clinician's point of view. An attempt was, therefore, made to see if the rat test could be modified to give rapid results. The intraperitoneal route was used for rapid absorption of the hormone. Several tests were done and the rats examined 2-4 hours after injection. To begin with, known urines from pregnant and non-pregnant women were injected and it was found that (i) the entire quantity of 2-4 cc. was absorbed within 2-3 hours, (ii) pregnant urines gave a positive result and (iii) non-pregnant urines gave a negative result. A positive case showed congestion and enlargement of the ovaries. In order to test the validity of this rapid rat test simultaneously, the usual 24 hour's rat test was put up for the same sample. The results compared very favourably. This work is in progress.

XVIII. In Vitro Activity of Indian Medicinal Plants.

(MALOO).

For the purposes of this investigation two medicinal plants were selected : (1) *Punica granata*, (2) *Ocimum sanctum*—Black and White variety.

The watery extracts of the roots, leaves, flowers and stem skin were tried after removing the chlorophyll and adjusting the pH to 7.4 against the common Gram positive and Gram negative pathogenic organisms.

Antibiotic activity was tested by (1) The modified double plate method of W. D. Frost, (2) Cup assay method.

It was found that majority of the extracts of different parts of both the plants showed inhibition of practically all the organisms tested except *E. coli*. The extracts of the peel of the fruit of the plant *P. granata* showed inhibition of *V. cholera* in dilution of 1 in 10,000.

XIX. Anerobically Grown Typhoid Organisms in Widal Test.

(NARASIMHAM).

Salmonella typhi, when grown under reduced oxygen pressure, or in CO₂, produce a changed type of somatic antigen which OGAWA has termed as "Anerobic Antigen". He has also shown that strains grown anerobically agglutinated more strongly with sera from typhoid patients, than aerobically grown organisms. He used live suspensions. The use of live suspensions is not advisable for routine Widal tests and so it was decided to use killed emulsions of anerobically grown typhoid organisms with a view to seeing whether the agglutinability of the killed emulsions in Widal tests was enhanced.

S. typhi, 0901 strain was grown inside a desiccator with the air partially aspirated out. The culture was grown at 37° C for 24 hours and again sub-cultured till the 7th generation was obtained. This growth was emulsified in saline, killed by 0.25 per cent. formalin and standardised to the same opacity as the standard emulsions now in use. This new killed suspension of the 7th generation of the anerobically grown strain was put up for agglutination tests along with the routine Widal tests. A few preliminary results indicate an increased agglutinability with this new suspension with sera from typhoid cases. This work is in hand.

XX. Tropical Eosinophilia—An Experimental Study on Laboratory Animals.

(KUMARI COLAH AND MODI).

In the routine examination of blood cell counts, a large number of cases was detected with Eosinophil count of over 20 per cent. Apparently, these were cases of Tropical Eosinophilia.

This field showed dearth of data and it was decided to (a) study eosinophil counts of normal laboratory animals and (b) see the effect on eosinophil count after inoculation of material such as blood, urine, sputum, etc., from cases of Tropical Eosinophilia.

With the collaboration of Dr. B. D. Patel, Hon. Pediatrician, B. J. Hospital, Bombay, it has been possible to obtain material and conduct this research.

Initially normal blood picture of mice, guinea-pigs and rabbits was studied. Forty-four blood examinations were carried out in white mice (20), guinea-pigs (12) and rabbits (12). Total W. B. C. count in mice (3 months' old) varied from 5,000-10,000 cu. mm. with an Eosinophil count from 0-2 per cent.; in guinea-pigs (4-5 months' old) from 4,000-10,000 cu. mm. with Eosinophils 0-6 per cent. and in rabbits (6-8 months' old) 6,000-10,000 cu. mm. with Eosinophils 1-10 per cent.

The effect on these animals of injecting normal human blood, i.e. with normal Eosinophil count was studied in 10 mice, 10 guinea-pigs and 5 rabbits. Blood counts were done in all these animals before injection and subsequently on the first, second, fourth and the seventh days afterwards. None of the animals showed any evidence of any alteration either in the total W.B. C. or in Eosinophil count.

Whole blood from 24 cases of Tropical Eosinophilia, showing Eosinophilic count of more than 20 per cent. was collected and injected intraperitoneally (1 cc. diluted in saline) in the abovementioned animals. These animals were kept under observation from 10-20 days and their blood examined at short intervals.

In all the animals, there was no significant response in the form of rise in Eosinophil count. There appeared a slight increase in total W. B. C. count which was more prominent in guinea-pig than in rabbit and white mouse and was of a transient nature.

Further work along these lines is in progress. It is proposed to see the effect of inoculation of sputum and urine of cases of Tropical Eosinophilia on the blood counts of the animals with a view to seeing if the causative principle of Tropical Eosinophilia is present in blood, sputum or urine of such cases.

XXI. Serodiagnosis of Syphilis—A Comparative Study.

(SAMANT)

This study started in 1952, was continued during the year under report. The work was done on the lines indicated previously. Comparison of the three flocculation tests was made on 883 samples. Of these 883 samples, 791 showed complete agreement with all the three tests and the rest, i.e., 92 sera showed conflicting results.

In 337 samples of sera, the syphilitic status of the patient was known. The results of these cases are analysed below.

Syphilitic Status		Number of samples	Result		V.D.R.L.	Kahn	M.K.R.
Primary	...	143	Negative	...	46	58	49
			Doubtful	...	0	9	10
			Positive	...	97	76	84
Secondary	...	154	Negative	...	25	36	35
			Doubtful	...	1	7	0
			Positive	...	128	111	119
Late or Tertiary	...	40	Negative	...	9	12	10
			Doubtful	...	0	2	0
			Positive	...	31	26	30

From the above data, it will be seen that in all stages of the disease, V.D.R.L. test showed the maximum number of positive, least number of negative and doubtful results.

XXII. Use of dyes in serodiagnostic tests for syphilis.

(SAMANT)

Different dyes have been used as sensitisers in flocculation tests for syphilis. Berger evolved a technique and used Victoria Blue 4 R and claimed that with his technique the results obtained were as good, if not better than, with Kahn or Wassermann tests. With the development of V.D.R.L. test, it was decided to see (a) whether the dye test could be compared with a more sensitive V. D. R. L. test, and (b) if other dyes could be used as well. Several dyes were used. The sera under test were simultaneously put up for Kahn and V. D. R. L. tests as well as with the various dyes under test for purposes of comparison. The results are tabulated below :—

Table showing the results of Dye test as compared with Kahn and V. D. R. L. Test for Syphilis.

Dye used	Dye Test			Total Sera Tested	Kahn Test			V.D.R.L.		
	+	+	—		+	+	—	+	+	—
Victoria Blue	...	171	16	273	459	149	23	287	175	4 280
Janus Green	...	170	16	273	459	149	23	287	175	4 280
Acraflavin	...	207	11	289	507	198	20	289	209	9 289
Brilliant Green	...	212	15	280	507	198	20	289	209	9 289
Fuchsin	...	216	15	276	507	198	20	289	209	9 289
Methylene Blue	...	226	13	268	507	216	22	269	237	1 269

Blue worked just as good sensitisers as Victoria Blue 4R and Janus Green. With the dyes, the results came almost up to the results obtained by the V. D. R. L. Test. Consequently, it is suggested that the Dye Test may be used as a screening test with great advantage. It is emphasised that the results are better read under the low power of the microscope. From the analysis of the data not given here, it is clear that the best results were obtained with Methylene Blue. Further work is in hand.

XXIII. Preservation of rabies virus by Freeze-drying.

(DOGRA).

The work in connection with the preservation of rabies virus by lyophilization, started in 1951, was continued and finalized during the year under review. Several determinations of titre of rabbit and mouse fixed rabies virus preserved in lyophilized state were made. Whereas there was an initial fall of titre after "freeze-drying" the brain pools that were satisfactorily freeze-dried and sealed in vacuum retained their potency for 12 to 24 months (Rabbit's Brain) and 4 to 12 months (Mouse Brain). Modern methods of freeze-drying at -50° to -70° C are being attempted to achieve better results, so that the initial lowering of titre may be reduced and the keeping qualities of the preparation increased.

XXIV. Immunity response to Semple's anti-rabic vaccine in Man.

(DOGRA).

A study of immunity response in man after Semple's anti-rabic treatment was undertaken in 1951. This was made possible after preservation of mouse-fixed virus by freeze-drying was accomplished in this laboratory. With this method a test virus of a constant titre was made available. A technique for neutralization test was worked out. Several samples of human blood from cases treated with Semple's vaccine treatment were taken and tested for the presence of viricidal bodies during 1951, 1952 and 1953. Altogether 50 samples were examined. The results are tabulated below.

Table showing Results of Mouse Protection Test with Human Sera from cases before and after Treatment with Semple's Anti-rabic Vaccine.

●Before treatment	After 1-3 months' treatment	After 4-6 months' treatment	After 7-9 months' treatment	After 12 months' treatment.
1. (5) 0/5	.. 1. Class III (1) 5/5	.. 1. Class III (7) 5/5	.. 1. Class II (13) 5/5	.. 1. Class III (25) 4/5
2. (6) 0/5	.. 2. Class II (2) 5/5	.. 2. Class III (12) 5/5	.. 2. Class II (22) 5/5	.. 2. Class III (26) 2/5
3. (9) 0/5	... 3. Class II (3) 5/5	... 3. Class II (17) 4/5	.. 3. Class II (41) 5/5	.. 3. Class II (27) 5/5
4. (10) 0/5	.. 4. Class III (4) 5/5	.. 4. Class II (20) 5/5	.. 4. Class II (42) 4/5	.. 4. Class II (28) 1/5
5. (14) 0/5	.. 5. Class II (8) 5/5	.. 5. Class II (21) 5/5	.. 5. Class III (43) 5/5	.. 5. Class II (29) 2/5
6. (18) 0/5	... 6. Class III (11) 5/5	.. 6. Class II (34) 5/5	.. 6. Class II (44) 5/5	.. 6. Class III (30) 4/5
7. (23) 0/5	.. 7. Class III (13) 5/5	... 7. Class II (35) 5/5	.. 7. Class II (45) 5/5	.. 7. Class II (31) 5/5
8. (24) 0/5	.. 8. Class III (16) 5/5	.. 8. Class II (36) 5/5	.. 8. Class II (46) 5/5	.. 8. Class II (32) 5/5
9. (39) 0/5	.. 9. Class II (19) 5/5	.. 9. Class II (37) 5/5	.. 9. Class II (47) 4/5	.. 9. Class II (40) 5/5
10. (48) 0/5	.. 10. Class II (33) 5/5	.. 10. Class III (38) 5/5	.. 10. Class II (50) 5/5	.. 10. Class III (49) 5/5

Nominators=Number of mice survived.
Denominators=Number of mice used.

ed complete absence of viricidal bodies those from treated cases with Class II and Class III treatments showed circulating viricidal bodies for almost 12 months after treatment.

Whereas the above data gave satisfactory proof of immunization in man with Class II and III treatments and of its long duration, it was necessary to find out whether immunity response to Class II and Class III treatments was the same in the initial stages of immunization. Neutralization tests were performed on the samples of blood before treatment, on the 14th day and the 28th day afterwards. The results are tabulated below.

Table showing results of Mouse Protection Test on Human Sera collected in the initial stages of the Immunization with Semple's Vaccine.

Serial No.	Patient's Number	Class of treatment	Results.			Remarks.
			1st sample.	2nd sample.	3rd sample.	
1	5023	II	0/5	4/5	5/5	Test virus used was freeze dried mouse brain Pool VII with LD ₅₀ titre of 10-7-50.
2	5046	II	0/5	5/5	5/5	Test virus dilution used 10-4.
3	5075	II	0/5	5/5	5/5	Sample I obtained before treatment. Sample II obtained on the 14th day after starting treatment.
4	5953	II	0/5	5/5	5/5	Sample III collected on the 28th day after starting treatment.
5	5027	III	0/5	2/5	5/5	
6	5050	III	0/5	5/5	5/5	Class II treatment 5×14 cc.
7	6535	III	0/5	4/5	5/5	Class III treatment 10×14cc.
8	6384	III	0/5	5/5	5/5	

Nominators=Number of mice survived.

Denominators=Number of mice used.

Semple's Vaccine in Class II and III treatments, the immunity response is the same. It would appear that Class III treatment was unnecessary.

It is proposed to make quantitative study of pooled sera from Class II and III cases at different stages of immunization after Semple's treatment with a view to confirming the above findings.

XXV. Antibiotic properties of Indigenous Plants.

(VAIDYA).

A number of plants were selected from among those popularly used by those practising indigenous medicine. Fresh plants were obtained and stored in a refrigerator. Extracts from plants were prepared in cold and hot water and alcohol. The antibiotic property was tested by the cup-plate method as this method showed advantages over the other methods such as—Double plate method, Ditch method, Drop-on-plate method and filter-paper disk method.

So far seventeen plants have been tried. In most cases all the available parts of the plants were extracted and tested. Most extracts showed no antibiotic activity except a few alcoholic extracts which showed some antibiotic activity against *St. aureus*. In the case of *Vernonia anthelmintica* seeds cold water and alcoholic extracts showed antibiotic activity. Attempts are being made to isolate the active principle from the seeds of this plant. The work is in progress.

XXVI. Studies on Influenza Viruses.

(SOMAN AND DIGHE).

(A) Isolation of Virus Strains.

In the beginning of the year 1953, Influenza occurred in epidemic proportions in U.S.A., Canada, U.K. and on the European Continent and reached as far as Egypt. The possibility of its further spread to India was kept in mind and with this in view, circulars were issued in Bombay to heads of various institutions and general practitioners, requesting them to supply material for virus isolations and blood specimens for virus identification; the response, however, was extremely poor. Still forty-nine throat-washings were tested for virus isolations during the year and a haemagglutinating virus could be isolated from two of them. One was lost during its second passage in eggs due to heavy fungal contamination. The other one, however, was well adapted to eggs and was identified as A Prime strain (Mainkar). This was later confirmed by the World Influenza Centre in London, as A-Prime (Liverpool subtype) and will be labelled as A/Bombay/1/53. The strain was recovered from one of the staff (Shri Mainkar) of this Institute, who gave history of mild attack of cold, muscular pains and headache of three days duration. The newly isolated strain was peculiar in that in the first 3-4 passages in eggs, it killed the chick embryos but afterwards behaved like any other well-adapted strain.

Haemagglutination inhibition tests were carried out on 12 paired sera and only one showed a distinct fourfold rise in antibody titre to influenza B-Strain, Lee. Repeated attempts to isolate the strain from throat-washing of this case (Thosar), however, were not successful.

(C) *Purification and Concentration of Influenza Viruses.*

This could be achieved by a number of methods such as (1) the process of adsorption to and elution from red cells; (2) centrifugation at high speed in cold and (3) chemical precipitation. Only the first two methods were tried in preliminary experiments. In the first method, Francis and Salk's technique was followed—adsorption with 2% fowl cells carried out in cold overnight and elution at 37° c. for 2½ hours next day. By this method, a six to ten-fold concentration could be easily achieved and a major portion of the inert egg protein eliminated. The method is valuable where virus is to be purified and concentrated in large amounts as for the purpose of preparation of influenza vaccines. In the second method, only small amounts of virus could be concentrated at a time by spinning it down in a refrigerated centrifuge for about two hours at 10,000 r. p. m.

(D) The standard strains and the local strains were maintained in regular egg passages and were utilised in preparing antisera by immunization of roosters.

XXVII. Further studies on the Laboratory diagnosis of small-Pox.

(SETHI AND SOMAN).

In view of the small number of small-pox cases investigated last year, the work was continued in this year and thirty-three cases, from the presumptive to the crusting stage, were studied. Presumptive diagnosis was made available to medical authorities after microscopic examination of stained smears within a few hours, but final diagnosis was made only after virus was isolated on the chorioallantois in 3 days.

The complement fixation test using the vesicle fluid as unknown antigen was carried out in 9 cases of small-pox and 3 cases of chicken-pox. The results were obtained in twentyfour hours and were confirmed by egg inoculation and further corroborated by clinical observation. Pending the preparation of hyperimmune anti-vaccinia rabbit serum, human sera from convalescent cases of small-pox were used. The titre of such sera was found to be low and varied from case to case. The anti-vaccinia rabbit hyperimmune serum has been prepared during this year and will be used in future. The study of the laboratory methods available for the diagnosis of small-pox and its differentiation from chicken-pox has yielded encouraging results, as it helped to make a final and correct diagnosis in three cases referred to by the Port Health authorities and the Superintendent, City Fever Hospital, Bombay.

XXVIII. Isolation of Herpes Virus.

(SOMAN AND SETHI).

The virus of Herpes Simplex was isolated from three cases diagnosed clinically, by growing it on the chorioallantois and by injecting mice and rabbits. On the chorioallantois, the lesions produced were pinpoint and opaque and much smaller in diameter than the vaccinia virus and the central necrosis was absent. Proper adaptation to the C. A. Membrane required a number of egg passages. Scrapings from these lesions showed elementary bodies in smears when stained by the usual technique.

Mice inoculated intracranially showed signs and symptoms of infection of herpes in 2-3 days and by three serial passages the incubation period could be reduced to 24 hours.

Intracranial inoculation of a rabbit with herpes material resulted in rise of temperature 104° F to 106° F on the 7th day, with typical signs and symptoms such as convulsions and tremors and peculiar circular movements of the animal. On sacrificing the animal, its brain was removed for histological study. On scarification of the cornea, the rabbit exhibited severe keratitis with purulent discharge. Smears made from scraping the cornea revealed the presence of deeply stained bodies similar to those of chickenpox in scanty numbers. Hardly any intranuclear inclusion bodies could be seen.

The histological study on the rabbit cornea, rabbit and mouse brain is under-way.

XXIX. Studies on street virus of Rabies.

(SOMAN AND KHEMANI).

(a) It is generally accepted that the animal suffering from rabies at the time of its death shows presence of negri bodies in the brain and excretes virus in the saliva. But this is not always so. With a view to find out whether the virus in the brain and salivary gland go hand in hand, specimens of brain and salivary glands from the rabid or suspected rabid animal were examined for the presence of virus either by detection of negri bodies or by mice inoculation.

Twenty nine specimens, brain and glands, from each animal were subjected to the above tests with the following results.

Brain + S. gland +	Brain + S. gland —	Brain — S. gland —	Brain — S. gland +	Total.
8	8	13	0	29

harbouring virus in brain need not be always infective to other animals through its saliva. But the reverse would not be true, as the presence of virus in salivary glands was invariably accompanied by its presence in the brain.

(b) Those salivary glands which proved positive for the presence of street virus were further titrated in mice for their infectivity titre; only seven glands could be tested this way. All these glands were stored in neutral glycerine and kept frozen at -20°C . Storage of material at -20°C in neutral glycerine did not affect appreciably the infectivity titre for at least 4 months. This was shown by repeatedly titrating the same gland material in mice. Perhaps they would remain longer without being affected if the glycerine used is replaced by fresh stock every 4 months. Although the period of storage varied, the method of storage remained the same for all glands tested. They were titrated at varying intervals depending on the availability of mice. The results are presented below.

Serial No.	Date of collection.	Period of storage		Infectivity L. D. 50 titre in mice
		Months	days	
15	23rd February 1953 ...	4	4	10-5.5
23	2nd April 1953 ...	2	25	10-4.
24	6th April 1953 ...	5	27	10-2.
29	7th April 1953 ...	5	17	10-4.2.
46	23rd June 1953 ...	3	6	10-3.0.
50	14th July 1953 ...	2	15	10-2.0.
81	19th November 1953.	1	2	10-3.69.

The results clearly suggest that the infectivity titre of the virus in salivary glands varies from case to case. Thus the chances of catching rabies infection depended not only on the severity, the extent and the location of the bite but also on the virulence of the virus strains which would vary from animal to animal. It is now proposed to test the infectivity titre in mice as soon as the glands are received.

XXX. Studies on avianized rabies vaccine.

(SOMAN AND KHEMANI).

The flurry strain which had only 4 egg passages last year went into further 10 egg passages during the year under report; the L. D., 50 mouse infectivity

Batch No.	Before lyophilisation L. D. 50 titre in mice.	After lyophilisation L. D. 50 titre in mice.
11	$10^{-4.0}$	10^{-2}
12	$10^{-3.5}$	$10^{-2.5}$
13	10^{-3}	$10^{-2.42}$
14	$10^{-3.53}$	$10^{-2.25}$

The protection test was carried out in Guinea pigs 350 grammes in weight and they were actively immunised with lyophilised flurry vaccine Batch No. 11 and at the end of three weeks were challenged by street virus salivary gland suspension diluted 1 in 20, infectivity titre $10^{-5.5}$. No protection was observed since it was found later, that the titre after lyophilisation had fallen to $10^{-2.0}$ and probably most of the live virus in that vaccine was dead. Many more protection tests on successive batches of lyophilised vaccine would be necessary before it is accepted as a suitable immunising agent. A prime necessity of maintaining a standard street virus for challenge was felt which, when used in certain dilution in protection experiments, would bring down 70 per cent. control animals. The work of testing street virus strains for this purpose is proceeding. On securing a street virus strain suitable for challenge, further batches of vaccine would be tested.

XXXI. Further observations on the Biological Test for the Diagnosis of Rabies.

(SOMAN AND KHEMANI).

The observations reported in 1952 on the value of biological test in the diagnosis of rabies were too small; hence they were extended to the year 1953 in which 89 specimens were examined for the diagnosis of rabies, out of which 6 were unfit for examination, due to decomposition. Out of 83 specimens, 37 were reported positive showing Negri bodies either in sections or smears. 36 brain specimens having been received in glycerine were available for mice inoculation. A comparative study of the impression technique for detection of negri bodies and the biological test in mice used in the diagnosis of rabies has again confirmed the previous observation that correct diagnosis of rabies in 7 cases would have been missed if biological tests were not carried out. In a recent case although microscopic examination was reported to be positive by us, the animal inoculation failed to produce any evidence of rabies. This case is still under study.

(VIJAY SINGH).

As a result of repeated potency tests, it was observed that most of the batches of Rockefeller Foundation yellow fever vaccine received here in 1946, had retained the requisite level of potency after 7 years of cold storage at a temperature of 0-4° C. Some of them were found to have kept up their full potency so far.

XXXIII. Post-Vaccinal Reactions.

(VIJAY SINGH).

Out of 10,099 inoculations carried out during the year, no cases of post-vaccinal reactions were reported.

XXXIV. Investigation on the Amino Acid Contents of Casein Hydrolysate for Intravenous use.

(THADANI AND DUTTA).

The hydrolysate of casein prepared at this Institute is being extensively used in patients suffering from protein deficiency due to various causes. The nutritive value of any hydrolysate of proteins depends on the amounts of essential and non-essential amino acids present in such preparations. An investigation was undertaken to determine the amino acid contents of the Casein Hydrolysate prepared by the partial acid digestion of Casein. Most of the amino acids were estimated microbiologically and only in a few instances chemical methods were used.

It was found that on an average Casein Hydrolysate contains 1.0 per cent. total nitrogen, 0.56 per cent total amino nitrogen and 9.0 per cent total solid.

The percentage of the different amino acids present in the hydrolysate calculated on the basis of total solid are as follows :—

Arginine 4.1 ; Phenyl alanine 5.0 ; Methionine 2.44 ; Threonine 5.8 ; Histidine 2.33 ; Lysine 5.5 ; Leucine 8.0 ; Valine 8.5 ; Tryptophane 0.32 ; Glutamic acid 24.4 ; Cystine 0.33 ; Tyrosine 3.3 ; Aspartic acid 0.22 ; Proline 5.9 ; Serine 4.4 ; Glycine 1.9.

A large number of vitamin preparations, which were received for analysis, contained folic acid incorporated with proteolysed liver extract with or without yeast or stomach extract, in combination with thiamine, riboflavin, pyridoxine and other members of the B-Complex and ascorbic acid; not infrequently combined with such inorganic salts as those of ferrous or ferric iron, sodium, potassium, copper etc. A general survey of the results of estimation of folic acid in such preparations revealed that the vitamin did not remain stable in most of them. Further study is undertaken to find out the applicability of different methods to the assay of folic acid in these type of preparation and the stability of the vitamin under different conditions.

XXXVI. A study of the Pharmacological Properties of Chaksine—An Alkaloid Derived from the seeds of cassia Absus Linn.

(TAMHANE AND DUTTA).

In anaesthetised cats, Chaksine Chloride (1mg./kg.) produced fall of blood pressure which was not blocked by either Anthisan (5mg./kg.) or Antistine (5mg./kg.). On the isolated guinea pig's intestine, Chaksine antagonised the contractions produced by histamine or acetylcholine. The inhibiting action of acetylcholine on the isolated spontaneously beating auricles was reduced by Chaksine. The drug relaxed isolated duodenum of the rabbit suspended in Tyrode solution. Chaksine has a weak "curari-like" action on the isolated rat diaphragm. On the perfused (with Locke's solution) vessels of the rabbit's ear, Chaksine produced constriction.

XXXVII. A study on the Stability of Vitamins in Oral Preparations—Effect of Vitamin A, Liver Extract, Alkaloids and Enzymes on Thiamine Hydrochloride.

(MEHTA, ANANTHANARAYANAN AND DUTTA).

The stability of thiamine hydrochloride in different mixtures containing Vitamin A, crude liver extracts (oral), pepsin, Takadiastase and alkaloids was studied. The preparations containing Vitamin B₁ with the liver extract and Vitamin A were kept (i) at 0–5° C, (ii) in an atmosphere of nitrogen, and (iii) in contact with air. The latter two preparations were kept at room temperature (25°–30° C). It was observed that thiamine lost about 25 per cent. of its potency at 0–5° C in one year. During the same period, in the sample kept at room temperature, thiamine had almost completely (95 per cent.) deteriorated. Thiamine, when kept in an atmosphere of nitrogen, only lost to the extent of 30 per cent. of its potency. Pepsin and Takadiastase produced some effect on the stability of aneurine but strychnine and quinine had no adverse effect.

XXXVIII. Placental Transmission of 4 : 4¹ Diamino Diphenyl Sulphone (DDS) in the Rabbits.

(LELE AND DUTTA).

The treatment of leprosy with 4 : 4¹ diamino-diphenyl sulphone (DDS) has now passed beyond the experimental stage and the drug has established itself as one of most useful in leprosy. The drug is known to be toxic if the dose is not kept within the limit of safety. In view of the fact that DDS is being used in pregnant lepers and because of its toxic nature, a study was undertaken to find out whether the drug passes from maternal to foetal circulation and whether it has any adverse effect on the fecundity and gravidity.

Six rabbits were fed with DDS 100 mg./Kg. daily, starting immediately on the day of mating and continuing till the day of delivery. Each animal, on an average, received about 3 g. DDS in one month. They did not show any untoward symptoms during the entire period of pregnancy. The litters, after the birth, appeared to be normal in weight, appearance and development like the control series. The concentration of DDS in the maternal blood and in the bloods of the new ones, born from their respective mothers were almost identical. The concentration of DDS in the maternal blood varied from 2.7 to 3.7 mgm per cent and in the young one's blood from 2.9 to 3.8 mgm. per cent. This experiment demonstrated the ability of DDS to pass freely through the placental barrier.

The above observations should be confirmed in human beings as the duration of pregnancy is not only prolonged but the treatment with DDS may extend for a long period.

XXXIX. Chemistry of the Schiff Bases of Sulfonamides.

(SARDESAI AND SHIRSAT).

During the course of synthesising numerous alkylidene and arylidene Schiff bases of the potent sulfonamides for investigation of their pharmacological and bacteriological properties (Report of the Haffkine Institute for the year 1952), a number of interesting observations regarding the chemical behaviour of these compounds have been made. Some of these observations are given below in brief.

The behaviour of Sulphathiazole towards the various aliphatic aldehydes was found to be different from that of Sulfadiazine and Sulfamerazine. Thus the condensation products of Sulphamerazine and Sulphadiazine were found to be insoluble in hydrochloric acid and soluble in sodium hydroxide as expected. The condensation products of Sulphathiazole with the aliphatic aldehydes were, however, found to be insoluble both in alkali and acid, suggesting thereby the participation of the Sulphonamido group as well as that of the aromatic amino group of sulphathiazole in the reaction. The condensation product of sulphathiazole with glyoxal, however, was found to be soluble in alkali and insoluble in hydrochloric acid.

these conditions, sulphadiazine furnished different condensation products, depending upon the alcohol used in the reaction, suggesting thereby the participation of the alcohol in the reaction. Other sulphonamides did not show such behaviour but furnished only one condensation product, whatever the alcohol used as medium in the reaction. It was found that the different condensation products of sulphadiazine with formaldehyde, obtained by employing different alcohols in the reaction, could all be converted first into simple Schiff bases and then into Sulphadiazine by heating with water for a suitable period. It was, therefore, interesting to study whether Sulphadiazine and formaldehyde would furnish a simple Schiff base if reacted in aqueous medium and it was found that such a reaction did take place. Sulphathiazole furnishes identical products by interaction with formaldehyde irrespective of whether the reaction is carried out in aqueous or alcoholic medium. The behaviour of sulphamerazine is similar to that of sulphathiazole.

Since sulphathiazole furnishes alkali and acid insoluble products when reacted with aliphatic aldehydes, it was interesting to study the nature of compounds obtainable by its interaction with aromatic aldehydes. It was, however, interesting to note that while the aliphatic aldehydes always furnished acid and alkali insoluble condensation products with sulphathiazole, whatever the molecular proportions of the reactants employed; the aromatic aldehydes in general furnished simple Schiff bases which were soluble in alkali and insoluble in acids. If, however, an excess of benzaldehyde or of cinnamaldehyde is employed in the reaction and if the heating period is extended to several hours, the corresponding condensation products are insoluble in alkali and insoluble in acid in both the cases. These alkali insoluble and acid insoluble products could be converted into alkali soluble and acid insoluble compounds by heating with alkali. It was, however, noted that the alkali soluble condensation product of cinnamaldehyde and sulphathiazole is rather unstable and gets converted into an alkali insoluble product on standing at room temperature or by heating for a short period. Condensation products of sulphamerazine, sulphadiazine and sulphaguanidine with the various aromatic aldehyde were found to be simple Schiff bases, under various reactions conditions tried.

XL. Chemistry of O-Hydroxy Sulphonamides.

(SARDESAI AND SHIRSAT).

During the course of synthesis of o-hydroxy sulphonamides for a study of their bacteriological properties, (Report of the Haffkine Institute for the year 1952), some interesting observations were made regarding the chemistry of these compounds. Thorpe and Williams (Biochem. J., 35, 61-65) isolated two condensation products by the interaction of 2-acetoxy-4-acetamino benzenesulfonyl chloride with ammonia. They were able to identify one of these condensation products as 2-hydroxy-4-acetaminobenzene sulphonamide while they could not determine the constitution of the other product (m. p. 235°C). - We have been able to confirm the results of Thorpe and Williams

has also been established by an independent synthesis. Since, such a finding suggests the migration of the acetyl radical of the acetoxy group at 2-position of the sulphonylchloride and its subsequent reaction with the free amino group of the sulphonamide, it was of interest to study the reaction of this sulphonylchloride with various other amines, and in the case of aniline, p-chloro-aniline and p-nitro-aniline, it was found that these amines do get acetylated when reacted with the sulphonylchloride and furnish the corresponding acetyl derivatives in addition to furnishing the corresponding 2-hydroxy-4-acetamino-benzene-sulphonamides.

In the case of some of the heterocyclic amines, e.g. 2-aminothiazole, 2-aminopyrimidine, 2-amino-4-methylpyrimidine, 2-amino 4 : 6-dimethylpyrimidine and guanidine, it was found that when these amines are reacted with 2-acetoxy-4-acetaminobenzene sulphonylchloride, an alkali and acid insoluble product is obtained in addition to the formation of the corresponding 2-hydroxy-4-acetamino-benzenesulphonamides. The acid and alkali insoluble product thus formed was found to be the same whatever the amine used in the reaction. The identity of this product is being further investigated.

XLI. Antimalarial Activity of Sulphonamides.

(SARDESAI AND SHIRSAT).

The various N¹-N¹-disubstituted sulphonamides synthesised as possible chemotherapeutics for the treatment of intestinal infections, (Report of Haffkine Institute for the year 1950), were also tested for their antimalarial activity against *P. Berghei* infection in mice and it was found that although the compounds are active as a class, none of these surpassed the activity of sulphadiazine. The introduction of an isopropyl group at the N¹-position of the various sulphonamides did not affect the antimalarial activity of these compounds to any appreciable extent. Among the most active of these compounds found was N¹-p-amino-phenyl sulphanilamide. Its quinine equivalent was found to be 15 but it was found to be less active than sulphadiazine. The antimalarial tests were carried out by Shri L. D. Siddons, Malaria Inquiry, I. C. M. R., at the Department of Chemotherapy, Haffkine Institute. A detailed report of this work has been published in the Indian Journal of Malariology, Vol. VII, No. 1, March, 1953.

XLII. Chemotherapy of Bacterial Infections— Synthesis of N¹-Benzimidazolyl Sulphanilamides and Metanilamides.

(BAPAT AND SHIRSAT).

Numerous N¹-benzimidazolyl derivatives of sulphanilamides and metanilamides have been synthesised.

During the course of the above work, it was found that the procedures as reported in literature for the preparation of arylbenzimidazoles do not furnish appreciable yields of 2-arylbenzimidazoles and, therefore, the reaction conditions had to be suitably modified to obtain the required compounds in good

molecular proportions. From the various experimental data collected during the course of the work, it has been observed that in the case of dinitro compounds one of the nitro group enters the 6-position of benzimidazole while the second nitro group enters the 2-aryl radical.

The structures of various 2-aryl-6-nitrobenzimidazoles have been proved by alternate methods of synthesis.

A number of 2-aryl-6-chlorobenzimidazoles have been synthesised as possible chemotherapeutic agents by reacting 4-chloro-*o*-phenylenediamine with several aliphatic and aromatic acids.

The antibacterial activity of these compounds is being investigated.

XLIII. Effect of Different Levels of Dietary Yeast in the Production of Hepatic Necrosis.

(RADHAKRISHNA RAO, TARA KUNDAJI, KRISHNAN AND GOVIND).

It has previously been reported that in this laboratory it has not been possible to produce regularly hepatic necrosis in rats either by dietary deficiency of Vitamin E, S-amino acids or simultaneous deficiencies of both. Recently McClean and Best (1952) have suggested that experimental hepatic necrosis can be produced in rats only at an optimum level of yeast; both higher and lower levels of yeast disavouring the production of the hepatic lesions. In order to ascertain whether there is any optimum level of dietary yeast which produced necrosis in the livers of experimental animals, preliminary experiments were carried out as described below :—

36 young male albino rats of the Haffkine inbred strain were divided equally into 6 groups with respect to litter mates, weights, etc. and fed 'yeast necrogenic diet' with yeast levels varying from 20 to 60 per cent. The rest of the diet was similar to that used in our previous experiments, except that alcohol and ether extracted Brewer's yeast (Squibb's) was used as the source of yeast instead of dry yeast of Burgoyne and Co., and instead of arachis oil at a level of 0.5 ml/rat/day, lard of Genuine Pork Products (Central Dairy Farm, Aligarh, U. P.) was substituted at the same level. The animals were fed this diet up to a period of 120 days. After 60 days on the diet they were killed at regular intervals and the liver studied histologically.

Results.—The liver sections of animals fed 60 per cent. yeast showed normal lobular pattern; the parenchymal cells showed normal histological appearances. Occasionally, there were traces of fatty infiltration of the parenchymal cells. When the yeast level was at 50 to 40 per cent., the liver sections showed similarly occasional fatty change in the parenchymal cells, and vacuolation of the cytoplasm. At 30, 25 and 20 per cent. yeast levels, the liver sections

As these experiments are only of a preliminary nature, further work is necessary to confirm these results and to determine the critical level of yeast at which this massive haemorrhagic necrosis can be produced regularly.

XLIV. Amino Acid Composition of Human Milk.

(TARA KUNDAJI AND RADHAKRISHNA RAO.)

'Kwashiorkor' and 'Infantile biliary cirrhosis' are usually prevalent in children during the weaning period, i.e. when the breast-fed baby is abruptly transferred to predominantly starchy foods, such as 'cassava' or rice diets. Recently, Bigwood (1952) has stressed the fact that this might be due to a diminution in the sulphur supply to the body in a diet formed predominantly of carbohydrates. It has been widely recognised that the building of tissue proteins (particularly those of the skin and hair) involves a high demand for sulphur; the amino acid methionine stands at the centre of the metabolic traffic between choline, Vitamin B 12, cystine and creatinine. With a view to unravelling the deficiencies in children's diets, which are primarily responsible for liver diseases, it has been suggested by various workers that a complete amino acid analysis of the milk of mothers, obtained from various economic social strata of the society will yield reliable data regarding the position of particular amino acids in infant nutrition.

The amino acids were determined qualitatively by the circular paper chromatographic technique (Giri, K. V., Current Science, 20, 295, 51) in about 35 samples of human colostrum and transitional milk secreted 2 to 10 days post-partum. The samples were collected from normal as well as undernourished mothers in order to compare the pattern of amino acids in the milk proteins, particularly, the presence of the sulphur-amino acids, methionine and cystine.

Results.—On examination of the chromatograms of the acid hydrolysate of the milk samples, 10 spots were observed in the case of normal milk while only 6 were found comparable with the standard in case of samples of milk from undernourished mothers. Tryptophane and cystine were absent in all the samples, probably due to their destruction during acid hydrolysis. Threonine, arginine and phenylalanine spots were not seen in the undernourished mothers' milk samples. These observations require further study and the work is now in progress.

XLV. Influence of Fermented foodstuffs on fatty infiltration of the livers.

(WAGLE, S. R. AND RADHAKRISHNA RAO.)

Liver disease and its prevention are world-wide problems, especially over the tropical belt of the world, and there is a wide consensus of opinion that liver disease is found concomitantly with general malnutrition—a low intake of dietary protein associated with proportionately larger intakes of carbohydrate

numerous foodstuffs and beverages which undergo fermentation at some stage or other in their preparation and it has been widely speculated that micro-organisms might contribute appreciably to a restricted dietary. Fermented foodstuffs may also lessen the strain put on the digestive enzymes of the body, by dismutating the macromolecules of the diet to more assimilable forms; thus, improving the efficiency of utilisation of foods in digestive disturbances as found, for example, in Kwashiorkor. Recently Gyorgy (1952) has adduced evidence to show that 'fermentation of food may increase under special circumstances the biological value even of a low protein diet'. In India, people are used to taking fermented foods in one way or other. Dahi (fermented milk) is *sine quanon* of most Indian meals. Other items are 'Iddlee', 'Dosai', leavened bread, 'Dhokla' etc.

Experimental studies on the effects of supplementation of some fermented food-stuffs on fatty livers produced by high-fat-low-protein diets are in progress.

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Parcel, Bombay, April 7, 1954.

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