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REPORT
OF
THE HAFFKINE INSTITUTE

For the Year 1951

BY

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

BOMBAY

PRINTED AT THE GOVERNMENT CENTRAL PRESS

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

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1951.

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1951.

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.B.B.S., B.Hy. (Bom.), D.P.H. (R.C.P.S.), D.T.M. & H. (Lond.), B.M.S.(I), I/c Department of Clinical Pathology (Returned from deputation abroad and resumed duties on 30th April 1951).

Looked after the duties of the Director during the absence on leave of the permanent Director from 7th May 1951 to 5th June 1951.

Dr. A. K. Hazra, M.B.B.S. (Bom.), D.T.M. (Cal.), B.M.S. (I), Officiated as Assistant Director I/c Department of Clinical Pathology in addition to his duties, from 1st January 1951 to 29th April 1951.

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

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

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

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

Dr. P. J. Deoras, M.Sc. (Nagpur), Ph.D. (Durham). Joined the Institute as Assistant Director in charge Department of Entomology, on 22nd January 1951.

II. GENERAL REVIEW.

During the year under report the Institute continued to function as the centre for the manufacture and supply of vaccines, parenteral solutions and other biologicals for the whole of India generally and to the State of Bombay in particular. The increasing demands for our products from various States is a proof that the public has appreciated their efficiency and the guarantee of their purity and utility. The increased demand is also due to the low rates at which our products are sold than the market rates. The Institute also continued to function as an analytical laboratory for the enforcement of Drugs Act, 1940. The sales amounted to Rupees Nineteen Lakhs while the expenditure was Rupees Seventeen Lakhs during the financial year 1950-51.

This year the demands for plague and cholera vaccines remained almost the same as those of the last year in spite of the continued efforts to check these diseases by regular campaigns with the help of powerful insecticides and also other measures. It appears that the demands for these vaccines have reached a level where no further reduction is anticipated. This year also plague vaccine was supplied to two foreign countries, viz., Burma and Arabia.

Work directed towards the improvement of the T.A.B. Vaccine, reported last year, is being continued and it is hoped that the new vaccine may soon replace the present agar vaccine. It is further proposed to conduct trials in human beings.

The demands for antitoxins and sera continued as before to be much in excess to what could be met. It appears that the difficulties reported in our production, due to the epizootic lymphangitis, are overcome, as no further cases were reported. The stud farm at Pimpri requisitioned last year for housing healthy equine stock, proved to be of great help and now it is proposed to acquire the farm on a permanent basis. In view of the steps taken last year, it is heartening to report that the supply was maintained and no serious dislocation in the production of the sera and antitoxins was encountered. The stables in the Institute are now again occupied and the production work is in good progress.

The high quality of the antitoxins and sera prepared at this Institute has been bringing demands from the Defence Services as well as other States. Antivenene has been in constant demand from outside countries.

The Department of Chemotherapy continued the manufacture of sulphathiazole tablets and injectable solutions. The sale of the tablets to the private medical practitioners had to be temporarily stopped because the demands were high due to the low sale price and the manufacture had to be interrupted due to the irregular procurement of the raw materials from abroad, in spite of our efforts. During the year under review, various leprosy clinics in Bombay State were supplied with 133,136 ml. of sulphathione in 50 ml. bulb packings and in 2 ml. ampoules, and in addition 381,550 ml. of Oleum Hydnocarpus with Creosotum was also supplied. The vitamin tablets manufactured at this Institute have become very popular with the public in spite of the fact that they have not been advertised. Arrangements are being made to distribute these tablets in the city and the mofussil through proper agencies so that they could be easily available to the public. The Government and Municipal Hospitals are also buying large quantities of these tablets. Researches centered round the discovery of new antimalarials and agents against intestinal infections. The methods of large-scale preparations of some vitamins and para-amino salicylic acid are being investigated.

The work of the Department of Pharmacology has increased considerably as a result of gradual enforcement of the Drugs Act not only in this State but also in the other States of the Indian Union. The total number of samples tested during the year under review was 1284 as against 924 only in the previous year. Under the Drugs Act, 740 products were analysed. An investigation carried out in this Department conclusively

demonstrated that vitamin B₁ is very unstable in oral mixture specially in the presence of alcohol, iron and copper. As a result of this, numerous oral preparations containing thiamine hydrochloride with any of the above constituents are now being suitably modified by the manufacturers. Verification of claims made by a number of people in connection with the prevention of alcoholic fermentation of Neera without altering its physical and chemical properties have so far not been substantiated.

In view of the increasing importance attached to the problem of prophylaxis in human and canine rabies, the demands on the Department of Virus Diseases for rabies vaccine continued to be on the increase and rabies vaccine was supplied to 134 centres. In April 1951, Dr. D. W. Soman, Assistant Director in charge of Clinical Pathology, returned to the Institute from his Fellowship studies on virus diseases under the Rockefeller Foundation. During a period of one year he contacted the leading scientists in U.S.A. and Canada and England and visited their laboratories and institutions and studied recent developments and techniques. Since his return he was entrusted with the work of organisation of the Influenza Centre at the Haffkine Institute and to carry out a Q fever survey, both being under the auspices of the World Health Organisation. Scientific Equipment worth about \$ 4,000 was supplied to the Institute by the World Health Organisation as a free gift in connection with the development of the Influenza Centre. Dr. Soman was permanently transferred to the Department of Viruses including Rabies on 20th December 1951.

Since the appointment of Dr. P. J. Deoras on 22nd January 1951, to the post of Assistant Director, Entomology Department, he has been able to tackle a number of new problems and start new investigation in the control of plague with special reference to Pyrethrum.

The Department of Clinical Pathology has continued the good work of giving diagnostic aid to various hospitals in the City and free diagnostic aid in case of infectious diseases and has also started work as a reference laboratory for venereal diseases. In view of Dr. Soman's transfer to the Department of Viruses including Rabies, Col. Dogra was permanently transferred to the Department of Clinical Pathology from 20th December 1951.

The Blood Bank has continued the supply of dried plasma to the hospitals and medical practitioners in the city and mofussil, but the supply of blood for this purpose is still coming primarily from jails and not from voluntary donors. The demand for the parenteral solutions like glucose saline and casein hydrolysate etc., is increasing year by year and has become a good source of additional revenue. Every help is being given to facilitate the work of the new Department of Nutrition which is housed in this Institute, but now administratively separated from the Institute.

Apart from its own regular and scientific activities the Institute had some full-time research workers who carried out research work under specific research grants obtained from the Indian Council of Medical Research, the National Institute of Sciences of India and the Government of Bombay. There were three I.C.M.R. Enquiries. Usually the problems tackled by the scholars are those which are of interest to the Institute.

During the year under report our Librarian, Shri M. V. Kamat, was deputed by Government to U.S.A., for training in Medical Librarianship under a Fellowship Grant from the Rockefeller Foundation, U.S.A. During the period of nine months he visited various libraries of cities and universities in U.S.A. and U.K. to study their working methods, planning and equipment with a view to reorganise the Haffkine Institute Library on modern lines.

III. DEPARTMENT OF VACCINES.

During the year under review the demand for Plague and Cholera Vaccine was slightly lower than last year while that for T.A.B. Vaccine remained practically the same.

7.9 million millilitres of Plague Vaccine and 5.7 million millilitres of cholera vaccine were issued during the year 1951 as against 9.1 million millilitres of plague and 6.3 million millilitres of cholera vaccine in 1950. The demand for T.A.B. Vaccine was constant at about 3 million millilitres for both these years. The total quantity of vaccines issued during the year 1951 is about 13.9 million millilitres.

The D.D.T. Campaigns in the State for control of Malaria and use of D.D.T. as a general insecticide at the time of epidemics in other States has mainly been responsible for lower demands of plague and cholera vaccine as reported last year. Demands for the vaccines were received from all parts of the country and for plague vaccine from some foreign countries. The provincewise distribution of plague vaccine is detailed below:—

A. Plague Vaccine.

During the year under report the quantities of Plague Vaccine issued are detailed below:—

Months.	Quantity in millilitres.		
January	...	...	10,19,480
February	...	...	58,149
March	...	...	5,34,150
April	...	...	2,88,625
May	...	...	77,547
June	...	...	13,150
July	...	...	15,350
August	...	...	58,412
September	...	...	11,12,830
October	...	...	19,57,330
November	...	...	27,23,880
December	...	...	55,215
Total...	...	...	79,14,118

Issue of Plague Vaccine from the Institute since its beginning in quinquennial periods given below will show the very great increases for its demand during recent years:—

Years	Quantity in millilitres
1898-1901	8,601,123
1902-1906	10,366,611
1907-1911	10,752,786
1912-1916	13,702,098
1917-1921	20,003,682
1922-1926	21,607,908
1927-1931	17,787,729
1932-1936	19,909,176
1937-1941	20,078,811
1942-1946	44,528,208
1947-1951	65,757,991
Total	253,096,123

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:—

India	Quantities in millilitres
Bombay State	4,44,380
Madras State	1,19,405
West Bengal	50,200
Uttar Pradesh	64,13,190
Bihar	3,00,530
East Punjab	22,420
Delhi	1,265
Madhya Bharat	900
Madhya Pradesh	2,04,120
Saurashtra	154
Hyderabad Dn.	5,640
Mysore	3,290
Rajasthan	270
Vindhya Pradesh	19,950
Travancore	3,000
Kashmir	350
Kutch	25
Other Miscellaneous	29
Total	75,89,118

Foreign Countries.

Burma	3,00,000
Arabia	25,000
Total	3,25,000

B. *Cholera Vaccine.*

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

Months				Quantity in millilitres
January	...	...	...	1,73,170
February	...	...	...	44,884
March	...	...	...	37,961
April	...	...	...	77,921
May	...	...	...	4,39,338
June	...	...	...	14,14,274
July	...	...	...	3,23,275
August	...	...	...	11,08,350
September	...	...	...	14,37,875
October	...	...	...	5,73,552
November	...	...	...	1,53,520
December	...	...	...	91,730
Total				58,75,850

Less received back from Pandharpur
Municipality, Pandharpur (B.S.) ... 1,15,000

Actually issued ... 57,60,850

C. *T.A.B. Vaccine.*

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

Months				Quantity in millilitres
January	...	...	...	23,766
February	...	...	...	20,225
March	...	...	...	12,521
April	...	...	...	21,745
May	...	...	...	31,854
June	...	...	...	27,859
July	...	...	...	34,533
August	...	...	...	50,559
September	...	...	...	28,353
October	...	...	...	44,412
November	...	...	...	17,454
December	...	...	...	8,937
Total				3,22,218

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 11,000 flasks (1000 ml. in each) of Casein Hydrolysate Medium for plague and 9000 flasks (500 ml. in each) for cholera were prepared. For the preparation of T.A.B. Vaccine, 2,400 Roux bottles (200 ml. of agar in acid digest broth in each) were prepared.

2. Different types of media were prepared both for routine and research for the diagnostic and other departments of the Institute and for the Indian Penicillin Committee. For testing the sterility of vaccines, toxins, antitoxins and parenteral fluids, 55,000 bottles each of aerobic and anaerobic medium in acid digest broth were prepared. Of these about 2,500 of each were supplied to the Indian Penicillin Committee during the year.

3. The parenteral fluids section has, within five years, outgrown the original plans and promises to increase still further. During the year, demands from beyond the Bombay State could not be met with for lack of both staff and equipment. The following table gives the detailed figures of sales during the year:—

Sale of Glucose Saline etc. during 1951.

Serial No.	Name of Product.	Number of bottles sold.	Amount
			Rs.
1.	Glucose Saline ...	33,321	66,642
2.	5 per cent. Glucose Solution. ...	6,644	13,228
3.	Protein Hydrolysate ...	5,494	10,988
4.	25 per cent. Glucose Solution, 100 c.c. ...	4,112	2,056
5.	25 per cent. Glucose Solution, 500 c.c. ...	1,506	3,765
6.	Normal Saline ...	1,587	3,174
7.	Hypertonic Saline ...	608	1,216
8.	Sodium Sulphate, Isotonic. ...	452	904
9.	4 per cent. Sodium Citrate. ...	407	814
10.	Distilled Water ...	761	380-8
Total ...		54,892	103,227-8

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

1948	...	...	15,995	20,781
1949	...	...	31,718	46,542
1950	...	...	47,111	88,838

IV. Department of Antitoxins and Sera.

Routine production of antitoxins, sera, and toxoids proceeded as usual during the year. Usefulness of the Pimpri Farm was proved by the production of the annual average quantities of antitoxins and sera with about half the usual number of horses housed at the Haffkine Institute. The preliminary immunization was carried out at Pimpri, and the horses were brought to Bombay where they were fully ready for bleeding and harvesting of antitoxins and sera.

Efforts of the workers of the department, devoted to making available for human use immunizing agents highly potent but not producing any unpleasant reactions, were fully rewarded this year with the evolution of a new diphtheria toxoid PSFT. This new toxoid has been found, both in animals and in human volunteers, to be as potent as, if not more than, the alum precipitated toxoid, while in the absence of any reactions whatsoever caused by it, it has proved to be far superior to the APT. After completion of a few more experiments in the field, this new toxoid will be issued for use in place of the APT which had to be withdrawn due to bad reactions caused by it.

1. *Horses*.—The number of horses used in the production of antitoxins and sera during the year was 68. Besides these there were 42 horses undergoing preliminary immunization at Pimpri Farm at the end of the year. The horses were grouped as follows:—

Antitoxins.	Number of horses used.
1. Tetanus Antitoxin ...	33
2. Diphtheria Antitoxin ...	11
3. Anti-snake-venom Serum ...	18
4. Welchii Antitoxin ...	3
5. Anti-dysentery Serum ...	nil
6. Normal Horse Serum ...	1
7. Anti-human Serum ...	2
	68

2. *Preparation of Toxins and Toxoids*.—During the year, tetanus, diphtheria and Welchii toxins were prepared for hyper-immunization of horses. The reserve stocks of the other toxins sufficed during the year. A part of the diphtheria and tetanus toxins were converted into toxoids for immunization of horses. Portion of these toxoids was reserved for human use.

The following are the amounts of toxins and toxoids prepared during the year:—

Types of Toxins and Toxoids.	Quantity in litres.
1. Tetanus ...	336
2. Diphtheria ...	139
3. Welchii ...	36
4. Septique ...	nil
5. Oedematiens ...	nil
6. Shiga ...	nil
7. Tetanus Toxin converted to Tetanus Toxoid.	38
8. Diphtheria Toxin converted to Diphtheria Toxoid.	71

3. *Preparation of Antitoxins and Sera.*—During the year the total volume of processed antitoxins and sera made available for human use amounted to 653,100 ml.

The quantity of each type of sera was as follows:—

I. Concentrated Sera.

No.	Name.	Amount of concentrated serum in ml.
1.	Tetanus Antitoxin ...	333,500
2.	Diphtheria Antitoxin ...	162,200
3.	Antivenin ...	104,500
4.	Anti-gas Gangrene (Welchii) ...	Nil
Total		600,200

II. Natural Sera.

No.	Name.	Volume in ml.
1.	Normal Horse Serum ...	36,100
2.	Special Horse Serum (Anti-human Serum).	16,800
Total		52,900

4. *Lyophilization of Sera.*—Only the polyvalent anti-snake-venom serum was issued in lyophilised form during the year. The amount of the serum issued was 50,060 ml.

5. *Supply.*—The following quantities of antitoxins sera and toxoid were issued during the year under review:—

	Quantities supplied in millilitres.
1. <i>Tetanus Antitoxin</i> —	
(a) Prophylactic 3,000 I.U. in 2 ml.	2,63,072
(b) Curative 10,000 I.U. in 5 ml.	8,890
(c) Curative 20,000 I.U. in 10 ml.	1,08,760
(d) Highly concentrated 20,000 I.U. in 4 ml.	50,220
2. <i>Diphtheria Antitoxin</i> —	
(a) Prophylactic 1,000 I.U. in 2 ml.	1,430
(b) Curative 5,000 I.U. in 5 ml.	2,785
(c) Curative 10,000 I.U. in 10 ml.	45,970
(d) Highly concentrated 10,000 I.U. in 5 ml.	17,495
3. <i>Gas-gangrene Antitoxin</i> —	
(a) 3,000 I.U. Welchii Antitoxin in 10 ml.	56,740
(b) 9,000 I.U. Welchii, 4,500 I.U. Septique and 9,000 I.U. Oedematiens Antitoxin 15 ml.	5,535
4. <i>Anti-plague Serum, 20 ml.</i>	780

	Quantities supplied in millilitres.		
5. <i>Lyophilised Polyvalent Anti-snake-venom Serum,</i> 10 ml.	...	...	50,060
6. <i>Anti-dysentery Serum</i>			
(a) Natural, 20 ml.	...	...	840
(b) Concentrated 4,000 I.U. in 5 ml.	...	...	665
7. <i>Tetanus Toxoid.</i>			
(a) 10 ml.	...	...	910
(b) 5 ml.	...	...	90
(c) 1 ml.	...	...	881
8. <i>Diphtheria Toxoid.</i>			
(a) 5 ml.	...	...	nil
(b) 1 ml.	...	...	nil
9. <i>Lyophilised Schick Test Toxin for 25 tests</i>	...	...	nil
10. <i>Normal Horse Serum, 10 ml.</i>	...	...	1,490

6. *Other activities of the Department.*—The department undertook to test the potency of 16 samples of Tetanus Antitoxin and 7 samples of Diphtheria Antitoxin for the Drug Standardisation Section of the Haffkine Institute.

V. DEPARTMENT OF ENTOMOLOGY.

During the year technical assistance was given to a number of Government departments and private bodies with regard to the information on mites, annelid worms, cyclops, fleas and insects damaging clothes.

There were six enquiries with regard to this Institute design "Wonder trap" for rat control and they were duly attended to.

Two courses of training in Plague and Plague Preventive Measures were conducted for the Epidemic Medical Officers and Sanitary Inspectors appointed by the Department of Public Health, Government of Bombay and the Local Bodies. The first course was held in March 1951 for officers from Bombay and Rajasthan States and the Second one was held in September, 1951, for 17 officers of the Bombay State. One day demonstrations and lectures were given on plague control measures to students of six Medical Colleges and allied institutions.

Preparations were made to take part in four public exhibitions where the activities of this Institute were on view.

Nineteen experiments were done with fleas sent here for testing purposes. Large number of fleas were bred in the laboratory and experiments done with some pyrethrum formulations to test the pulicidal value.

Methyl Bromide in ampoules was tested to find out its rodenticidal property.

Investigations were carried out and report submitted on the feasibility otherwise of establishing fumigation centres to eradicate the fleas that may be imported into the City of Bombay in foodgrains coming by rail, land or sea from plague infected areas.

A. Snakes.

Twenty-seven snake specimens were identified which were sent to this Institute as reptiles involved in snake-bite cases in the City of Bombay.

The total number of snakes kept during the year was 1128 of which *Echis carinata* predominated. The total venom extracted during the year was as below :—

<i>Vipera russelli</i> (Russell's viper)	<i>Naja naja</i> (Cobra)	<i>Echis carinata</i> (Phoosa)	<i>Bungarus coeruleus</i> (Krait)
36.25 gms.	27.112 gms.	7.71 gms.	2.612 gms.

Demand for snake venom continued to be heavy even from the countries such as Brazil, U. S. A., Poland, Siam, U. K. and Hungary. Twenty-five educational institutions and a large number of public were shown round various sections of the Institute and snake-venom extraction.

Observations on the causes of snake mortality in cages were conducted. *Echis carinata* fed on *Lacerta viridis* lived longer than when kept on milk and eggs. Preparations to start an open air snake farm and a scheme of research on Indian Poisonous Snakes were finalised.

B. Examination of Rats from Bombay City and Port Trust Area.

On an average, 2,810 rats per day were received at this Institute from different wards within the municipal area. These were either trapped alive or collected dead in the various localities including the Port Trust Areas. Table No. I shows the number of such rats received each month and Table No. II gives the number of rats examined for suspected plague. Only the dead ones were dissected and examined for post-mortem signs of plague infection. The rodents were destroyed in an incinerator after careful examination. Table Nos. III and IV give the percentage of different species in the catch and Table No. V gives this data from the Port Trust Area.

C. Examination of rats from steamers and other sources.

During the year, 22 dead rats and 12 smears were received from different places in Bombay State. All these were negative to plague and are detailed in Table No. VI. The Port Health Officer, Bombay, sent 194 killed rats and they were found to be negative to plague. Of these 157 were *Rattus rattus*, 14 *Rattus norvegicus*, 5 *Gunomys kok* and 18 *M. dubius*.

25 rats were received from the City of Bombay through other agencies or private persons. All these were negative to plague.

390 live rats were supplied, as shown in Table No. VII, to various Colleges for teaching zoology and research.

This rat work gives a valuable information to municipal authorities on plague epidemiology and gives the first signal of any plague epizootic in any part of the city which ultimately helps to control the spread of plague epidemic in time.

TABLE I.—Showing rats received at the Institute from Bombay Municipality during 1951.

Month.	Alive.	Dead.	Total.
January	... 86,149	1,287	87,436
February	.. 79,741	1,097	80,838
March	... 85,764	1,117	86,881
April	... 79,636	1,186	80,822
May	... 71,191	943	72,134
June	... 75,262	889	76,151
July	... 87,171	980	88,151
August	... 92,747	934	93,681
September	... 82,565	877	83,442
October	... 89,280	871	90,151
November	... 87,290	769	88,059
December	... 97,151	831	97,982
Total	... 10,13,947	11,781	10,25,728

TABLE II.—Showing dead rats examined for plague during 1951.

Month.	Dead rats examined.	Found plague infected.
January	... 1,121	Nil
February	... 972	Nil
March	.. 998	Nil
April	... 1,013	Nil
May	... 857	Nil
June	... 827	Nil
July	... 883	Nil
August	... 881	Nil
September	.. 836	Nil
October	... 781	Nil
November	... 672	Nil
December	... 753	Nil
Total	... 10,594	Nil

In addition to dead rats, 216 live rats from the plague infected localities and 9,187 live rats from the Bombay Port Trust area were examined during the year. The total number of live and dead rats examined is 19,997.

TABLES III, IV AND V.

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

Species.	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ...	18,486	21.4	17,441	21.9	18,779	21.8	16,407	20.6	13,151	18.5	11,750	16.6
<i>Rattus norvegicus</i> ...	15,909	18.6	14,467	18.1	14,614	17.3	13,718	17.2	12,003	16.9	13,371	17.8
<i>Gunomys kok</i> ...	82,507	37.7	31,731	39.8	33,745	39.3	30,613	38.5	28,362	39.8	31,104	41.3
<i>Bandicota malabarica</i> ...	963	1.1	730	0.9	548	0.6	487	0.6	426	0.6	726	1.0
<i>Mus dubius</i> ...	9,615	11.2	6,335	8.0	7,890	9.2	7,915	9.9	7,321	10.3	6,667	8.8
<i>Suncus caeruleus</i> ...	8,669	10.1	9,037	11.3	10,188	11.8	10,496	13.2	9,923	13.9	11,644	15.5
Total ...	86,149	100.0	79,741	100.0	85,764	100.0	79,636	100.0	71,191	100.0	75,262	100.0

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

Species.	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens</i> ...	246	19.1	214	19.5	194	17.4	202	17.0	267	23.3	179	20.1
<i>Rattus norvegicus</i> ...	317	24.6	314	28.7	284	25.4	262	22.1	182	19.3	242	27.2
<i>Gunomys kok</i> ...	546	42.4	444	40.5	519	46.5	544	45.9	408	43.3	405	45.6
<i>Bandicota malabarica</i> ...	6	0.5	...	...	...	...	1	0.0	...	...	...	...
<i>Mus dubius</i> ...	6	0.5	...	...	1	0.0	4	0.3	...	...	1	0.1
<i>Suncus caeruleus</i> ...	2	0.2	1	0.0	24	2.2	16	1.4	7	0.7	9	1.0
<i>Putrid</i> ...	164	12.7	124	11.3	95	8.5	157	13.3	79	8.4	53	6.0
Total ...	1,287	100.0	1,097	100.0	1,117	100.0	1,186	100.0	943	100.0	889	100.0

trapped alive by Bombay Municipal Corporation, Bombay, during 1951.

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
15,819	18.1	18,787	20.3	18,169	22.0	18,608	20.8	20,804	23.8	25,452	26.2	2,13,653	21.1
13,503	15.5	14,365	15.5	12,230	14.8	14,424	16.2	13,576	15.5	15,579	16.0	1,67,759	16.5
33,360	38.3	30,642	33.0	20,649	37.1	31,811	35.6	27,550	31.6	22,865	30.8	3,71,939	35.7
847	1.0	661	0.7	499	0.6	619	0.7	660	0.8	1,063	1.1	8,234	0.8
11,031	12.6	15,158	15.3	10,555	12.8	10,883	12.2	12,078	13.8	13,993	14.4	1,19,446	11.8
12,611	14.5	13,134	14.2	10,463	12.7	19,930	14.5	12,622	14.5	11,194	11.5	1,32,916	13.1
87,171	100.0	92,747	100.0	82,565	100.0	89,280	100.0	87,290	100.0	97,161	100.0	10,13,947	100.0

rats found dead in the City of Bombay, during 1951.

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
246	25.1	296	31.7	303	34.5	331	38.0	263	36.8	339	40.8	3,100	26.3
234	23.9	211	22.6	193	22.0	205	23.5	157	20.4	174	20.9	2,775	23.6
396	40.4	370	39.6	340	38.8	244	28.1	226	29.4	239	28.8	4,681	39.7
...	...	1	0.1	...	...	...	...	...	...	...	...	8	0.1
7	0.7	3	0.3	...	...	1	0.1	6	0.8	1	0.1	50	0.2
5	0.5	1	0.1	6	0.7	9	1.0	10	1.3	4	0.5	94	0.8
92	9.4	52	5.6	35	0.4	81	9.3	87	11.3	74	8.9	1,093	9.3
980	100.0	934	100.0	877	100.0	871	100.0	769	100.0	831	100.0	11,781	100.0

B. EXAMINATION OF RATS FROM THE

These rats were received along with those of the other wards of the Bom- given in previous tables. A separate record of these rats, giving the number the Public Health Commissioner.

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

Species.	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
<i>Rattus rattus rufescens.</i>	375	41.7	446	49.5	270	31.0	452	51.0	321	39.8	279	34.7
<i>Rattus norvegicus</i> ...	186	20.9	218	24.2	232	25.5	153	17.3	146	18.1	162	20.2
<i>Gunomys kok</i> ...	235	26.1	162	18.0	249	28.5	168	19.0	189	17.3	163	20.3
<i>Bandicota malabarica</i> ...	...	...	6	0.6	6	0.7	3	0.3	3	0.4	8	1.0
<i>Mus dubius</i> ...	48	5.3	31	3.4	68	7.8	31	3.5	114	14.1	131	16.3
<i>Suncus caeruleus</i> ...	54	6.0	39	4.3	17	1.9	79	8.9	84	10.4	60	7.5
Total ...	900	100.0	902	100.0	872	100.0	886	100.0	807	100.0	803	100.0

BOMBAY PORT TRUST DOCKS,

bay Municipality, and have been already accounted for in the monthly totals and percentage of the different species, is also maintained at the instance of

of rats caught alive from the Bombay Port Trust Area in 1951.

July		August		September		October		November		December		Whole year	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
374	47.8	383	48.3	422	51.8	379	50.1	317	45.1	58.5		4,540	45.8
86	11.0	122	15.4	175	21.5	123	16.2	122	17.4	120	13.5	1,837	18.6
100	12.8	110	13.9	134	16.5	106	14.0	1.5	15.0	114	12.8	1,785	18.0
13	1.7	4	0.5	5	0.6	2	0.3	2	0.3	19	2.1	71	0.7
163	20.8	106	13.3	39	4.8	88	11.6	76	10.8	59	6.6	954	9.6
46	5.9	68	8.6	39	4.8	59	7.8	80	11.4	58	6.5	723	7.3
782	100.0	793	100.0	814	100.0	757	100.0	702	100.0	892	100.0	9,910	100.0

TABLE VI.—Showing the different localities in the Bombay Province from which smears and rats were received for examination during 1951.

Name of Town.	No. of smears.	No. of rats.
Thana	...	4
Kalyan	...	7
Ambernath	...	4
Vaitarna	...	3
Bijapur (Bagalkot)	1	...
Islampur	2	...
Badlapur	...	1
Dhanduka	...	1
Pen (Kolaba)	2	...
Borivli	...	1
Navsari	...	1
Nadiad	7	...
Total	12	22

TABLE VII.—Bombay City Rats supplied to different institutions during the year 1951.

Names of the Institutions.	St. Xavier College.	Khalsa College.	Siddarth College.	Wilson College.	Ram Narain Ruia College.	Bombay Natural History Society.	Royal Institute of Science.	Topiwalla National Medical College.
No. of rats supplied ...	72	8	10	129	81	10	62	18
Total No. of rats supplied.	390							

VI. DEPARTMENT OF CLINICAL PATHOLOGY.

A. Bacteriological Section.

This section examined clinical material from various Government hospitals and dispensaries in the city and the mofussil places in the State. In addition, private medical practitioners contributed a good number of samples for diagnostic work. Free diagnostic aid was given to all practitioners in infectious diseases such as diphtheria, dysentery, cerebro-spinal fever, enteric fever, typhus fevers, cholera, plague, malaria, etc. The typhoid scheme sanctioned by Government in 1947, continued its operation during the year 1951 and the Institute doctor attended 814 calls from private practitioners. The scheme is becoming very popular, as larger number of doctors are taking advantage, which primarily helped the notification of this disease from the public health aspect.

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

No.	Nature of Examination.	No. of samples examined.	No. of positive samples.
1	Wassermann Test	... 4,536	1,573
2	Kahn Test	... 2,589	684
3	Widal Test	... 2,167	800
4	Blood cultures for enteric group of organisms.	4,151	558
5	Agglutination tests for typhus fever.	4,206	204
6	Agglutination tests for Brucellosis.	2,186	12
7	Material for T. B. by smear, culture and animal inoculation test.	671	118
8	Throat swabs for diphtheria	... 189	52
9	Stools for routine Examination	... 1,057	432
10	Stools for culture (Including rectal swabs).	809	16
11	Blood films for malaria	... 409	17
12	Material for plague	... 11	1
13	Friedman test and rat test for pregnancy.	386	165
14	Urine for cultural examination	... 113	30
15	Urine for routine examination	... 978	...
16	C. S. F.—routine and culture	... 414	...
17	Blood counts	... 1,363	...
18	Material for detection of gonococci.	56	...
19	Material from medico-legal cases	... 27	...
20	Rideal Walker Test	... 30	...
21	Autogenous vaccines	... 56	...
22	Semen examination	... 50	...
23	Agglutination test for leptospirosis.	37	...
24	Material for Paul-Bunnell Test	... 10	...
25	Material for myelogram examination.	22	...

5 strains of dysentery organisms, 1 of *B. typhosus* and 4 of *salmonella* group were isolated, on routine cultural examination of stools.

The maintenance of various species of poisonous snakes for the purpose of extraction and collection of snake venom was transferred to Entomology Section, this year.

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

No.	Reagents.	Amount supplied.
1	Blood grouping sera	5,719 ml.
2	High titre sera	729 ml.
3	Killed suspensions for Widal and Weil-Felix tests.	1,19,920 ml.
4	Kahn antigen	3,964 ml.
5	Amboceptor	108 ml.
6	W. R. Antigen	650 ml.
7	R. V. Venom Solutions :	
	1:1000	377 ml.
	1:3000	135 ml.
	1:10,000	71 ml.
8	Cobra Venom Solutions :	
	5 M. U.	84 ml.
	10 M. U.	86 ml.
	15 M. U.	68 ml.
	20 M. U.	47 ml.
	25 M. U.	63 ml.
	Complete box of graduated doses ...	131 boxes.

B. Section of Public Health Chemistry and Bacteriology.

This section mainly examined samples of water for their fitness for drinking, sanitary and industrial purposes; it also examined other miscellaneous samples.

The following table gives a summary of the various examinations carried out during the year 1951:—

Sample examined.	Nature of examination.	No. of samples examined.
Water.	Fitness for drinking purposes ...	70
(Water aerated).	Fitness for drinking purposes ...	1
Water.	Fitness for sanitary and industrial purposes ...	27
Marmite.	Bacteriological examination ...	1
Peptone.	Fitness for bacteriological purposes.	1

C. Histo-Pathology Section.

During the year under review, 1151 pathological specimens, received from different hospitals in this State and a few from outside the State of Bombay were examined. These include brains from suspected rabid animals, neoplasms, gynæcological conditions, tuberculosis of different organs and some rare and interesting pathological specimens. Besides, 251 specimens from experimental animals were examined to study the morbid histological changes. These were mostly from the different research enquiries conducted at the Institute. It would appear that a total of 1,402 specimens were subjected to histological examination.

The classification of the specimens of tissues received from hospitals in the city of Bombay and outstations and examined at the Institute during the years 1949, 1950 and 1951 is set out in the following table:—

Classification of pathological specimens received for examination.

	1949	1950	1951	1951 Per cent.
(1) Tissues found to be normal ...	42	55	143	12.4
(2) Tissues unfit for examination ...	66	88	107	9.3
(3) Brain tissues for Negri Bodies ...	118	93	52	4.5
(4) Miscellaneous Pathological conditions ...	457	504	609	53.2
(5) Tuberculosis ...	39	47	74	6.5
(6) Non-malignant neoplasms ...	114	73	74	6.5
(7) Malignant tumours ...	96	84	92	8.0
Total ...	932	944	1,151	100.0

It would appear that there has been a steady increase in the demands for Histopathological Examinations.

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

I. NEOPLASMS.

(a) Malignant:—

(1) Carcinoma Primary—

Adenocarcinoma	...	...	9
Carcinoma Simplex	...	...	34
Epidermoid Carcinoma	...	...	22
Papillary Carcinoma	...	...	1
Scirrhus Carcinoma	...	...	1
Squamous-cell Carcinoma	...	...	4

(2) Carcinoma Secondary

...	...	3
-----	-----	---

(3) Sarcoma—

Fibrosarcoma	...	...	4
Melanotic Sarcoma	...	...	1
Osteogenic Sarcoma	...	...	1

(4) Malignant Lymphoma—

Hodgkin's Sarcoma	...	...	4
Lymphosarcoma	...	...	2

(5) Malignant Hypernephroma

...	...	1
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(6) Chorion Epithelioma

...	...	1
-----	-----	---

(7) Seminoma

...	...	2
-----	-----	---

(8) Teratoma

...	...	2
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(b) Non-Malignant Neoplasms:—

Cavernous Angioma	...	...	1
Cappillary Hemangioma	...	...	1
Chondroma	...	...	1
Colloid Goitre	...	...	7
Cystadenoma	...	...	1
Fibroadenoma	...	...	9
Fibrolipoma	...	...	2

Fibroma	...	...	5
Fibroma Cellular	...	...	1
Fibrous Epulis	...	...	2
Fibromyoma	...	...	27
Giant Cell Tumour	...	...	1
Lipoma	...	...	1
Melanoma	...	...	1
Neurofibroma	...	...	4
Osteofibroma	...	...	1
Osteoma	...	...	1
Pseudomucinous cystadenoma	...	...	1
Lymphoblastoma	...	...	1
Mixed Parotid Tumour	...	...	3
Rhinoporiidiosis.	...	...	3

II. TUBERCULOSIS IN DIFFERENT ORGANS.

Breast	...	...	3
Female Genital Tract	...	...	34
Intestinal Tract	...	...	5
Lymph Nodes	...	...	24
Other Tissues	...	...	8

Under Government of Bombay Scheme for training of Technicians, the following were given complete training in histopathological technique.

- (1) Shri J. V. Undavia.
- (2) Shri G. V. Huliyaalkar.

VII. DEPARTMENT OF PHARMACOLOGY.

Medical men are making constant use of a large number of drugs either simple or complex, the potency of which cannot be judged unless they are subjected to chemical, biochemical, and biological tests. The need of such tests is being well recognised in our country. This is evidenced by the fact that a very large number of drugs and other specimens were tested in the department during the year covered by this report than any of the preceding years. The total number of samples examined during 1951 is 1,284 as against 924 tested in 1950, i.e., an increase to the extent of 40 per cent. over the last year. The total included 740 samples analysed under the Drugs Act. The samples that were examined both on behalf of the State Governments and Commercial Firms included a variety of substances like pharmaceutical products, indigenous drugs, chemicals, vaccines, antitoxins, antibiotics, proprietary medicines, surgical dressings and glasswares. A condensed list of all the preparations analysed is given in the accompanying table. The figures given also include those samples which were sent here for preliminary testing before they were finally standardised.

About 53 per cent. of the drugs examined in the department were "Biological" and "Special products" (i.e. Schedule C and C (1), Drugs Rules, 1945). Many of these preparations could not be tested by chemical or physical means alone, and the potency of which could only be judged by biological experiments of each specimen by direct comparison with a stable standard. Such experiments were done on specially selected animals. We had to increase the breeding of animals in order to meet the demand for testing of biological and special products.

During the course of the year, a very large number of oral vitamin preparations, containing thiamine hydrochloride, riboflavin, ascorbic acid, nicotinic acid in association with salts of calcium, iron, copper, manganese, extracts of liver, spleen, stomach, etc., in various combination, were analysed here. Some of these oral elixirs contained as many as dozen ingredients. In many of these pharmaceutical preparations, the vitamin contents were found to be lower than the declared amount. Possibility was considered that some of the inorganic constituents mentioned above might have deleterious effect on the stability of the vitamins in oral preparations. An investigation carried out here for a year indicated that thiamine hydrochloride undergoes deterioration in aqueous solutions and such loss of potency is accelerated in the presence of ferri et ammon citras, ferrous sulphate, copper sulphate and alcohol (15 per cent). It was further observed that thiamine mononitrate which is reported to be more stable than thiamine hydrochloride behaves in the same way as the latter substance.

Owing to the limited facilities available in other places, demand for testing of parenteral preparations for the absence of pyrogenic substances has been very great. Two hundred and forty-seven injectable substances were examined out of which 17 contained pyrogenic matter.

Some of the injectable preparations like glyucose saline, normal saline, distilled water, calcium gluconate, etc., which otherwise would have been considered to be of standard quality failed to pass the test for clarity. These substances contained suspended particles.

Out of the five samples of Ergot of Rye assayed, the alkaloidal content in 4 specimens, was lower than the prescribed limit. It is significant to note that samples of the same batch of Ergot collected from different parts of the same container gave widely variable results.

Condensed statement of the results of analysis of drugs, chemicals, etc., during the year 1951.

Name of the product.	Total No. examined.	Standard quality.	Not of standard quality.
Acids (mineral)	4	2	2
Aletris Cordial (so called)	4	...	4
Antitoxins	12	9	3
Adrenaline	12	9	3
Absorbent Cotton Wool	11	1	10
Absorbent Lint	3	...	3
Absorbent Gauze	3	...	3
Ampoules (glass)	17	17	...
Aspirin or similar preparations	3	2	1
Belladonna Liniment	2	...	2
Belladonna Glycerine	2	...	2
Camphor in Oil	2	2	...
Chloroform	2	1	1
Calcium Lactate	2	1	1
Calcium Gluconate	15	7	8
Calcium Salts (other than mentioned).	6	3	3
Distilled Water	41	26	15

Name of the product.	Total No. examined.	Standard quality.	Not of standard quality.
Dihydrostreptomycin	2	1	1
Digitalis Tincture	25	12	13
Digitalis (other preparations)	4	1	3
Easton's Syrup	3	2	1
Ergot, liquid extract of	12	5	7
Ergot Rye	5	1	4
Ether	5	2	3
Emetine Hydrochloride	7	5	2
Enzyme preparations	6	...	4 2*
Ephedrine hydrochloride	11	8	3
Extract of Kurchi	2	...	2
Extract of Liquorice	1	1	...
Extract of Viburn	1	...	1
Glucose Saline	93	92	1
Glucose Solution	64	51	13
Gripe Water	30	10	20
Glycerine	3	3	...
Glucose (powder)	1	...	1
Ginger	2	1	1
Honey	5	3	2
Hormones (other than mentioned).	4	3	1
Hydrogen Peroxide	5	3	2
Hexamine	3	2	1
Insulin	8	8	...
Iron and Ammonium Citrate	12	3	9
Indigenous Drugs	10	6	4
Kaolin Powder	2	1	1
Lactose	4	1	3
Laxative tablets	6	6	...
Liniment of Iodine	1	...	1
Liniment of Turpentine	6	3	3
Liver Extract	4	2	2
Milk of Magnesia	7	4	3
Milk Protein	2	2	...
Nupercaine	1	1	...
Oil of Anisi	1	1	...
Oil of Cassia	2	...	2
Oil of Castor	2	2	...
Oil of Chenopodi	1	1	...
Oil of Cinnamon	1	...	1
Oil of Citronella	2	2	...
Oil of Chaulmoogra	1	1	...
Oil of Clove	3	2	1
Oil of Eucalyptus	15	7	8
Oil of Groundnut	16	11	5
Oil of Hydnocarpus	6	5	1
Oil of Mustard	1	...	1
Oil of Olive	5	3	2

*No opinion could be given.

Name of the product.	Total No. examined.	Standard quality.	Not of standard quality.
Oil of Peppermint ...	3	1	2
Oil of Pudina ...	1	...	1
Oil of Til ...	3	2	1
Oil of Turpentine ...	3	2	1
Ointments ...	17	7	10
Oxymel of squill ...	2	...	2
Other drugs ...	62	28	32 2*
Protein Hydrolysate ...	37	29	8
Procaine ...	2	2	...
Penicillin ...	30	24	6
PAS ...	7	7	...
Pyrethrum ...	3	3	...
Potassium Salts ...	4	4	...
Preparations containing morphine ...	4	3	1
Quinine and its preparations ...	30	14	16
Quinine containing mixtures ...	4	3	1
Quinine Ethyl Carbonate ...	3	3	...
Sedatives ...	21	11	10
Solution of Ammonium Acetate ...	3	3	...
Solution of Mercuric Chloride ...	2	1	1
Solution of Lead Subacetate ...	1	...	1
Solution of Strychnine Hydrochloride ...	1	...	1
Solution, Arsenical ...	2	2	...
Spirit of Quinine and Ammonia ...	9	7	2
Spirit of Nitrous Ether ...	2	...	2
Spirit Ammon Aromat ...	11	8	3
Saline Hypertonic ...	5	5	...
Saline Normal ...	14	8	6
Sulphanilamide and its derivatives ...	41	33	8
Sulfarsphenamine ...	1	1	...
Syrups of codeine, glycerophosphates, etc. ...	16	7	9
Santonin ...	2	2	...
Saccharine. ...	1	...	1
Sodium Bicarbonate ...	2	1	1
Sodium Sulphate ...	4	3	1
Sodium Salts (other than mentioned) ...	5	4	1
Salts of ammonium, magnesium, zinc and bismuth ...	7	6	1
Tea decoction ...	4	2	2
Thyroid ...	3	1	2
Tincture of Belladonna ...	5	2	3
Tincture of Cardamom (compound) ...	5	3	2

*No opinion could be given.

Name of the product.	Total No. examined.	Standard quality.	Not of quality.
Tincture of Cinchona Composita ...	5	3	2
Tincture of Camphor Composita ...	3	3	...
Tincture of Hyoscyamus ...	11	4	7
Tincture of Iodine ...	8	1	7
Tincture of Nux Vomica ...	6	1	5
Tincture of Strophanthus ...	1	1	...
Tincture of Ipecac ...	6	2	4
Tincture of Ginger ...	18	10	8
Tinctures (other than mentioned) ...	14	11	3
Vaccines ...	14	11	3
<i>Vitamin Preparations:</i>			
Vitamin A preparations ...	7	5	2
Vitamin B1 preparations ...	26	20	6
Vitamin B2 preparations ...	4	3	1
Vitamin C preparations ...	30	21	9
Vitamin E preparations ...	1	1	...
Folic Acid preparations ...	12	7	5
Niacin preparations ...	4	4	...
Vitamin Complex preparations ...	137	52	85
Cod and Shark Liver Oils ...	37	24	13
"Waterbury's Compound" (so called) ...	2	...	2
"Zephrol" ...	2	2	...
Total ...	1,284	786	494 4*

Sulphanilamide Paste.—The department prepared 350 pounds of paste containing 10 per cent., of sulphanilamide and oleum vitaminatum for distribution to hospitals, charitable institutions etc.

Research and other activities.—A summarised statement of the investigation carried out in this department is given in Section II of the report.

The stability of various vitamins in oral multivitamin preparations has been investigated. The study has indicated that both thiamine hydrochloride and mononitrate lose their potencies within a couple of months in presence of ferrous sulphate, ferri et ammonicitras and copper sulphate.

Symptoms of poisoning in man due to snake venom at some stages simulate that of acute effects of histamine on tissues. Experimental studies have demonstrated that Indian Cobra Venom is a potent liberator of histamine from rat diaphragm. In the concentration of 100ug/ml. of the venom, the tissue was nearly depleted of its histamine content in the course of 45 minutes.

Probably the most important advance in the chemotherapy of leprosy during recent years is the introduction of diamino-diphenyl sulphone (DDS) for treatment of the disease. Some pharmacological studies

*No opinion could be given.

specialy relating to its absorbtion in various tissues of the rabbit were carried out.

Investigation on Neera.—As many as 15 different substances suggested by various people were tested for their ability to prevent alcoholic fermentation in Neera without any way altering its physical or biological properties. None of the substances examined, could preserve Neera in its natural state for 72 hours.

VIII. DEPARTMENT OF CHEMOTHERAPY.

The regular manufacture of sulphathiazole, both in the form of tablets and injectibles, was continued during the year under review, and the demands of the various Government and Municipal Hospitals were duly met. The orders of the Government extending the sale of sulphathiazole tablets to private medical practitioners met with great success as evidenced by the magnitude of sale of sulphathiazole tablets. There was a very large demand for tablets due to the low selling price (Rs. 35/- per 1000 tablets of sulphathiazole) at which these tablets were sold by this Institute as compared with the prevalent market prices. Unfortunately, the supply to these private medical practitioners and private hospitals had first to be rationed and then stopped, to ensure regular supplies to Government Institutions. This step had unfortunately to be taken in spite of considerable manufacturing capacity of the unit, due to difficulties in procuring and maintaining adequate stocks of the raw materials required.

The department, during the year under review, supplied the following quantities of sulphathiazole:—

Sulphathiazole Tablets	2,713,525 tablets
Sodium Sulphathiazole Injectable ...	39,240 cc. + 11,560 cc. to Blood Bank

The department has now been successful in thoroughly standardising all the steps involved in the process of manufacture. The working methods, as well as the set up and the schedule of operations were worked out to augment the productive capacity in order to meet the ever-increasing demands of sulphathiazole. The factor which at present limits the production is the inadequate stock of raw materials in hand and unavailability of raw materials in time, the supplies of which have been irregular. This has resulted in the department being forced to reduce the rate of production. During this time, when the production was curtailed, the staff of the department was engaged in overhauling the equipment, which has been needing urgent attention and replacement, no separate engineering staff being provided to look after the equipment and machinery in the department. Trials by several improved processes are in progress with a view to increase the overall percentage yield and thus reduce the cost of manufacture of sulphathiazole.

The preparation of "Sulphetrone" injectible for the treatment of leprosy in State-aided hospitals was continued and a total of about 133,138 c.c. of this drug were supplied in 50 c.c. bulk packing and in 2 c.c. ampoules. Besides this 381,550 c.c. of "Oleum Hydnocarp with Cresotum" was processed and supplied in various packings to the various leprosy clinics in the State of Bombay.

The demand for the antiseptic solution "PEMON" prepared by the department showed slight increase over the last year. About 3900 lbs. of Pemon was prepared and supplied to various hospitals in the State of Bombay.

The preliminary investigations in the researches conducted for the preparation of Nicotinic Acid, Vitamin B₁ (Thiamine Hydrochloride), and para-Aminosalicylic Acid(PAS), having been completed, the standardisation of the methods to be adopted in the various stages are now underway.

A large number of compounds have been synthesised and submitted for testing their therapeutic activity in experimental infections. Further work in this direction is in progress.

Vitamin Tablet Section

The section continued the manufacture of various kinds of vitamin tablets as usual. In addition a good number of sulphathizole tablets were also manufactured.

In spite of the fact that a very small fraction of the public is at all aware of the availability of our vitamin products at this Institute (the same being not advertised), the daily sales seem to be shooting up at the Institute counter. The matter of making the products available to the public through the approved agency, at every corner of the city, and also in the district places in the State of Bombay, is under active consideration. It is hoped that discussion will have a definite shape within the next few months, extending the benefit of the facility to a large section of the public.

A large number of these vitamin tablets are also being consumed by the Government and Municipal hospitals in the city as well as within the Bombay State, and also by various local, and charitable dispensaries. The consumption figures in these hospitals and dispensaries are enough to point to the fact that activity is really of immense use to the Government and the public. Other requirements of these hospitals such as for sulphathiazole tablets and sodium sulphathiazole injectibles are also adequately met by the department.

A comparative table showing the manufacture of vitamin and sulphathizole tablets during the past years and the year under report is shown below:—

Kind of tablets.	Production during			
	1948	1949	1950	1951
.. Vitamin A-D ...	7,05,470	17,82,620	26,63,450	33,36,000
.. Vitamin B-C ...	7,35,030	17,91,010	34,30,250	44,60,600
.. Vitamin B-C No. 3 ...			35,560	2,11,550
.. Vitamin B ₁ ...	1,73,845	2,44,150	1,29,500	1,98,500
.. Vitamin B ₂ ...	36,900	69,290	57,400	79,600
.. Vitamin C ...	1,99,800	4,11,800	2,96,900	2,87,100
.. Acid Nicotinic ...	16,320	1,65,450	1,14,800	1,29,500
.. Sulphathiazole ...	1,08,000	8,46,655	11,73,000	27,82,000
	19,75,365	133,10,475	139,12,860	115,83,850

* Four million of each were prepared for the Government for free distribution to the famine-stricken area.

† These three million of each were supplied to the Ministry of Food, Government of India.

‡ Prepared as special tablets for special composition.

IX. DEPARTMENT OF BIOCHEMISTRY.

During the year under review the department continued the active and useful aid given to the hospitals and practitioners. The number of samples received showed a steady increase, the total number of analysis carried out being 4264 for the year. This comprised of :—

Blood examinations	...	...	...	2,170
Urine	...	...	...	179
Cerebrospinal Fluid	...	...	...	1,149
Body Fluids	...	...	...	87
Milk Samples	...	...	...	471
Special tests (such as Basal Metabolic Rate, Glucose tolerance test, Gastric analysis, Liver and Kidney Function Tests, Urinary 17-ketosteroids	...	...	...	208

The number of blood samples coming for analysis, particularly for estimations of Albumin, Globulin, Urea-N, Cholesterol, Hemoglobin, Sodium and Potassium have increased considerably during the year under review. Towards the end of the year the number of milk samples received showed a sudden leap and at this rate we expect to receive more than 1500 to 2000 samples of milk alone per year.

Improvements in analytical methods and manipulations are constantly tried and adopted wherever necessary with a view to combine simpler techniques with high and reproducible accuracy. Some of the major changes made have been reviewed in the Annual Report for 1950.

There is a great demand for the special estimations of serum sodium potassium and the 17-ketosteroids in urine and we are now in a position to meet this.

In addition to the routine work, we supply some special reagents whenever needed by outside hospitals. This includes particularly, the supply of Methylene Blue Solution for intravenous injections to the leprosy centres and anti-cyanide kit to the Public Health Department.

The department provided facilities for training three technicians.

X. DEPARTMENT OF VIRUS DISEASES
INCLUDING RABIES.

The department continued to (1) afford anti-rabic treatment to patients in the northern part of the island of Bombay, (2) prepare very large quantities of anti-rabic vaccine for issue to anti-rabic treatment centres, (3) assist in the diagnosis of rabies in animals by histological examination of brain tissues, (4) hold stocks of and conduct yellow fever inoculations with the assistance of the yellow fever diagnostic unit sanctioned by the Government of India and (5) conduct research in virus diseases.

(a) *Anti-rabic Treatment Centre at this Institute.*—During the year under report 6,459 persons applied for anti-rabic treatment; of these 5,865 completed the full course of treatment, 624 were "advice cases". In cases where there was a doubt about the condition of the dog inflicting the bite and the dog was available for observation the 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 an "advice case".

Study of statistics regarding patients who applied for and submitted to treatment presents interesting data. Detailed information in this connection is set out in Table I.

TABLE I.—*Data in connection with Rabies Treatment carried out at the Institute during 1947, 1948, 1949, 1950 and 1951.*

Details.	1947	1948	1949	1950	1951
1. No. of persons who applied for treatment.	4,688	5,125	5,413	5,473	6,489
2. Advice cases	602	802	775	539	624
3. Fully treated	4,086	3,775	4,620	4,934	5,865
4. Discontinued treatments ...	Nil.	548	18	Nil.	Nil.
5. Percentage of health returns .	95.14	96.15	97.20	95.00	Awaited.
6. Cases of Hydrophobia ...	Nil.	Nil.	Nil.	1	Nil.

(b) *Preparation of Anti-rabic Vaccine.*—Five per cent., Semple's phenolised sheep brain vaccine was prepared as usual and issued to various anti-rabic treatment centres, depending on this Institute for supplies, and to indentors from outside India. Anti-rabic treatments issued by months are shown in the accompanying Table II.

TABLE II.—*Number of anti-rabic treatments issued to different anti-rabic treatment centres during the years 1949, 1950 and 1951.*

Month.	No. of treatments for human use.			No. of treatments for veterinary purposes.			Canine Vaccine in doses.	
	Year 1949.	Year 1950.	Year 1951.	Year 1949.	Year 1950.	Year 1951.	Year 1950.	Year 1951.
January ...	842	971	1,075	82	47	40	8	11
February ...	753	1,401	1,232	43	32	42	10	9
March ...	711	996	1,202	43	75	59	2	7
April ...	1,127	1,084	957	95	43	55	17	21
May ...	825	1,125	1,228	66	47	66	5	22
June ...	1,069	913	938	54	69	54	...	9
July ...	808	1,028	1,068	88	32	63	5	13
August ...	969	971	1,095	67	55	76	1	2
September ...	1,147	1,048	880	72	54	58	8	10
October ...	736	778	1,330	33	46	39	1	3
November ...	1,134	991	1,405	36	37	54	1	10
December ...	993	768	1,022	41	63	40	20	16
Total ...	11,044	11,468	13,432	675	606	646	78	133

It would be observed that 13,432 treatments were issued during the year under report for human use and 646 for veterinary purposes. The figures emphasize the popularity and demand for Semple's Vaccine from this Institute for both human and veterinary purposes. Single dose Canine Vaccine—25 per cent. sheep brain emulsion—which was started only recently was also sold in larger quantities.

(c) *Antirabic Treatment Centres*.—The following new centres were opened during the year 1951, bringing the total of such centres dependent for vaccine on this Institute to 134.

- (1) C. M. S. Hospital, Lusadia, district Ahmedabad.
- (2) Civil Hospital, Himmatnagar.
- (3) Cantonment Hospital, Deolali.
- (4) Ramrejendra Sinhji Hospital, Limbdi.
- (5) Malji Dispensary, Broach.

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

TABLE III.—*Source of brains received from rabies from Bombay City, adjoining suburbs and outstations in 1951.*

				Positive.	Negative.	Unfit.	Total.
Bombay City	...	...	...	11	13	2	26
Suburbs	...	...	...	17	12	3	30
Outstations	...	—	...	17	12	3	30
Total				28	25	5	56

TABLE IV.—*Statement showing the classification of different animals with the result of brain examinations for rabies during 1951.*

	Animals.			Positive.	Negative.	Unfit.	Total.
Jackal	...	...	...	1	...	...	1
Bullocks	...	...	...	...	2	...	2
Goats	...	...	...	1	1	...	2
Cat	...	...	...	...	1	...	1
Dog	...	...	...	26	21	5	52

(e) *Yellow Fever Vaccine and Inoculation*.—The yellow fever diagnostic unit at the Haffkine Institute continued its work of yellow fever inoculations and testing of the potency of yellow fever vaccine stocked here on behalf of the Government of India. Several batches of the Rockefeller Foundation yellow fever vaccine were tested for potency during the year. The number of tests performed were 48. Repeated potency tests on several batches, have shown that the potency of the vaccine is retained for much longer period than what was expected. Some batches have, so far, been found to be potent for three to five years.

The number of persons immunized against yellow fever during 1951 are shown in Table V. These persons were air or sea passengers from Bombay proceeding to or passing through yellow fever epidemic areas. Figures for 1949 and 1950 are given for purposes of comparison.

TABLE V.

Month.				No. of persons immunized.		
				1949.	1950.	1951.
January	...	...	...	384	402	667
February	...	...	...	802	876	705
March	...	...	...	563	686	529
April	...	...	...	1,356	856	883
May	...	...	...	651	715	3,250
June	...	...	...	618	569	571
July	...	...	...	457	607	250
August	...	...	...	801	966	567
September	...	...	...	592	639	595
October	...	...	...	427	725	667
November	...	...	...	585	681	694
December	...	...	...	649	539	817
Total				7,885	8,261	10,195

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

Centre.	Quantity of vaccine Supplied in doses.	
	1950	1951
1. The Chief Medical Officer, Porbander ...	460	440
2. The Port Health Officer, Jamnagar ...	400	500
3. The Medical Research Officer, Ceylon ...	4,000	NIL
4. The Director-General of Medical Services Noa-Goa.	600	600
5. The Provincial Public Health Laboratory, New Delhi.	480	2,080
6. The King Institute, Guindy, Madras. ...	NIL	800
7. The All-India Institute of Hygiene and Public Health, Calcutta.	3,000	4,000
8. The Air-port Health Officer, Santa Cruz. ...	720	2,460
Total ...	9,660	10,880

(f) *Research Work connected with preparation of Rabies Vaccine.*—

(i) Random samples of Rabies Vaccine were assayed with Habel's Technique. Samples examined showed that the vaccine possessed a high degree of potency.

Three brews of single dose Canine Vaccine prepared were assayed. They showed a satisfactory level of potency.

(ii) *Keeping Qualities of Semple's Vaccine.*—This study was completed during the year. It was found that (a) Rabies Vaccine if not stored at 5-8°C, rapidly deteriorated in its potency; (b) soon after preparation the vaccine brews showed lower potency values, but all of them improved on storage of 4-6 weeks (six determinations) justifying the conclusion that Rabies Vaccine (Semple's) should be "matured" before use; (c) when stored at 5-8°C, the titre of the vaccine remained above the prescribed limit for 10-16 months justifying the extension of expiry period of Semple's vaccine from 6-8 months provided it is kept in cold storage; (d) potency value of vaccine varied from brain to brain suggesting that the vaccine made from several brains be pooled to get a satisfactory uniformity of titre.

XI. BLOOD BANK DEPARTMENT.

During the year under report, the work of this department shows a considerable increase over the last year. The demand for dried plasma has been on the increase especially from the mofussils. It was possible to meet this demand as a large number of prisoners offer blood donations in the prisons. However this demand for plasma may not be maintained at this level as plasma substitute like Dextran and P.V.P. are being introduced in the market and this department may have to extend its activities to manufacture of plasma fractions, which are also essential requirements in the modern treatment.

As usual all the blood samples were grouped and the 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 carried out in the department during the year under report.

3023 blood donors were screened by the CuSO₄ specific gravity method for their fitness to give blood out of which 1804 donors were rejected thus giving a rejection rate of 59.6%.

3365 blood samples were tested for Kahn test of which 696 were positive, i.e., 20.6 per cent.

8000 c.c. blood with high haemoglobin titre was supplied to the Diagnostic Department for preparation of Blood Grouping Sera.

The following statements show the work of the department during the year 1951:—

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

Centre.	Type of Donor.			No. of Blood Donors.	Total.
Haffkine Institute ...	...	Paid	...	1,217	2,633
		Voluntary	...	5	
		Jail donors	...	1,411	
Indian Red Cross Centre ...	...	Paid	...	742	747
		Voluntary	...	5	
		Voluntary	...	121	
Other Hospitals ...	...	Voluntary	...	121	121
Grand Total ...					3,501

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

	Product.					Quantity in litres.
Blood	...	...	...	...	...	890.250
Liquid Plasma	...	...	...	...	...	473.500
Dried Plasma	...	...	...	...	...	461.750

TABLE III—*Showing the Distribution of Plasma.*

	Nature.					Quantity in litres.	Bottles.
Sold	...	...	...	...	...	373.000	1,492
Free distribution	...	...	...	...	...	119.750	473
Total					...	492.750	1,971

The work in connection with the medicolegal serology could not make much progress as the necessary staff for it was not available.

The work in connection with the Rh factor has been on the increase. A paper on Investigations on the occurrence of Rh sensitization in Bombay was presented at the 8th Bombay Medical Congress held on 8th December.

The work on genetical distribution with reference to Rh subgroups is in hand.

The effects of repeated blood donation and regeneration of different constituents after donations of blood are being studied.

Studies on Dextran with special reference to the possibility of production of dextran from Nira are also being investigated.

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 researches 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. Following is the list of persons for whom training was given in the various departments during the year 1951:—

S. No.	Name.	Designation and Address.	Department in which trained.	Duration of training.
1	Shri Asanand Sharma, B.Sc.	Chemist i/c of the Tablet Section, Drug Research Laboratories, Jammu and Kashmir.	Department of Chemotherapy.	7th May 1951 to 15th May 1951.
2	Dr. D. R. Kulkarni, M.B.B.S. (Bom.), I.R.C.P. (Lond.) F.C.S. (Lond.).	Assistant Director (Public Health), Central Laboratory, Narayanguda, Hyderabad-Deccan.	Department of Vaccines, Clinical Pathology, Biochemistry, Blood Bank.	6th August 1951 to 27th October 1951.
3	Shri B. P. Desai, B.Sc. ...	Technician of Mr Coyaji from Poona.	Department of Clinical Pathology and Department of Biochemistry.	Six months from 15th October 1951.
4	Dr. G. A. Shirvadam ...	Medical Officer, C M S. Hospital, Lusadia, district Sabar Kantha.	Department of Virus Diseases including Rabies.	26th March 1951 to 31st March 1951.
5	Dr. S. N. Dalvi, M.B.B.S. ...	Medical Officer, Associated Cement, Cos. Ltd., at Khalari Factory.	Department of Virus Diseases including Rabies.	31st May 1951 to 6th June 1951.

XIII. CRITICS CLUB.

During the year, 48 weekly meetings of the club were held in the new lecture room on Fridays. At these meetings, abstracts from the current literature pertaining to various subjects were read. Eleven talks were given by members from the various departments in their own subjects as mentioned below. *

Two foreign scientists addressed the club on the following subjects:—

1. Dr. Miss C. Halde, Bacteriologist, Army Medical Research, Hawaii, on 21st June 1951. Medical mycology with reference to skin diseases in the Tropics.
2. Dr. Earnest Tierkel, W.H.O., Expert on Rabies, on 30th November 1951. Recent advances in antirabies work.

This club was host to Teaching Pathologists on 29th September 1951.

- *1. "Incretory activity in Invertebrates" on 2nd March 1951.
—Dr. P. J. Deoras.
2. "Reminiscences of my U. S. A. on 1st June 1951.
Tour"—Dr. D. W. Soman.
3. "Phase Theory"—Dr. P. J. Deoras. on 15th June 1951.
4. "Chemical approach to virology"—on 27th July 1951.
Dr. Mrs. A. Venkatraman.
5. "Review of some work on Diphtheria on 7th September 1951.
Toxoid done during current months"—Dr. D. C. Lahiri.
6. "Lyophilisation and Rabies Virus"—on 21st September 1951.
Lt. Col., J. R. Dogra.
- ✓ 7. "The Role of Entomology in the on 5th October 1951.
Control of a Plague Epidemic"
—Dr. P. J. Deoras.
8. "The role of some Phosphorylated on 26th October 1951.
Organic Compounds in bio-
logical reactions with special refer-
ence to those containing a high
energy phosphate band"—
Dr. Mrs. A. Venkatraman.
9. "The chemistry of cortisone with on 2nd November 1951.
special reference to its manu-
facture"—Dr. Mehta.
10. "My impression of U. S. A. Medical on 25th November 1951.
Science Libraries"—Mr. M. V.
Kamat.
11. "Motion Sickness"—Mr. A. I. Mehta. on 28th December 1951.

XIV.—MISCELLANEOUS.

Apart from the researches carried out by the members of the staff, certain scientific enquiries which were initiated many years back at this Institute by the Indian Council of Medical Research, New Delhi, continued to be conducted during the year 1951 also. They are still being continued and they are as follows :—

- | | | |
|---|-----|---|
| 1. Plague Researches | ... | Under Dr. P. M. Wagle, Director,
Haffkine Institute, Bombay. |
| 2. Enquiry on the Chemotherapy
of Malaria. | | Under Dr. K. Ganapathi, Assistant
Director, in charge Department
of Chemotherapy. |
| 3. Enquiry on the Role of Nutri-
tional Factors in Hepatic
Cirrhosis. | | Under Dr. M. V. Radhakrishnarao,
Assistant Director, Department of
Nutrition. |

In view of the excellent Laboratory facilities this Institute has, scientific bodies like the National Institute of Sciences, and the Indian Council of Medical Research offered certain scholarships and attached the following scholars to this Institute during the year 1951.

1. Shri A. M. Mehta, D. Phil.—National Institute of Sciences of India, Junior Research Fellow—Subject of Fellowship—Investigation on biles (from slaughter-houses in Bombay) with a view to search for 11 and 12 oxygenated steroids needed for synthesis of antiarthritis compounds related to cartisone.

2. Shrimati Shanta S. Rao, M.Sc., Indian Council of Medical Research Fellow—Subject of Fellowship—"Researches on the purification and chemical structure of immune protein and active immunising agents."

3. Dr. P. P. Lele, M.B.B.S.—Indian Council of Medical Research Fellow—Subject of Fellowship—"Pharmacological action of some sulphones with particular reference to their action in leprosy."

In addition to these, the following Research Scholars and Paying Scholars worked in the Institute during the year under report :—

1. Kumari Tara R. Kundaji, B.Sc., Research Scholar No. 1—Govt. of Bombay.
2. Kumari K. M. Pavri, M.Sc., Research Scholar No. 2—Govt. of Bombay.
3. Kumari R. S. Vakil, B.Sc., paying scholar.

XV.—INCOME AND EXPENDITURE.

Receipts.		Expenditure.	
	Rs.		Rs.
XXVII.—Medical—		38, Medical—	
Sale Proceeds of Sera and Vaccines.	18,92,297	H. Bacteriological Laboratories,	
Other miscellaneous receipts	21,018	H. 1 Haffkine Institute and	
		H. 2 Pasteur Institute.	
		Pay of Officers	1,94,774
		Pay of Establishment	3,88,623
		Allowances etc.	3,18,320
		Contingencies	8,23,037
		Research Scholarships	1,698
Total Receipts	19,13,315	Total Expenditure	17,26,452

N. B.—No expenditure was incurred under H. 2 Pasteur Institute this year.

Part II.

RESEARCH WORK.

1. MOUSE PROTECTIVE VALUE OF THE CRYSTALLINE ANTIGEN FROM P. PESTIS.

(Wagle and Habbu).

Immunogenic Value of Protein Fraction IA.—Baker et al (1947) have reported that when saline suspensions of plague organisms grown at 37°C were precipitated by acetone at—70°C, a precipitate was obtained which when repeatedly washed with acetone and dried *in vacuo* gave bacterial powder of high immunogenic value. Extraction of this powder with neutral saline yielded a water soluble antigenic component. This component was further fractionated with 0.3 saturation of ammonium sulphate at pH 7.0 to 7.5. This fraction is called by the authors "Fraction IA" and this they have found to be highly immunogenic. Dr. Karl Mayer of the Hooper Foundation sent us some "Fraction IA" for testing by our method.

The fraction was tested as follows : The powder was dissolved in water and 6 groups of mice each were given subcutaneously two equal doses at an interval of one week. The 1st group received 0.25 μ g; the 2nd 0.5 μ g; the 3rd 1.0 μ g; the 4th group 2.0 μ g; the 5th group 5.0 μ g; and the last group 10.0 μ g. Two weeks after the 2nd dose was given a challenge dose of 1020 organisms (100 m.l.d.) of a fully virulent strain of *P. Pestis* was given subcutaneously. The animals were observed for a period of 3 weeks.

The following results were obtained :—

Dose in micro-grams.						Number of organisms in challenge dose.	Controls.	50 per cent. mouse Protective Dose.
0.25	0.50	1.00	2.00	5.00	10.00			
*3/4	5/5	4/5	1/5	2/5	1/5	1020	5/5	1.75 micrograms.

*The upper figures denote the deaths and the lower figures the total number of mice used.

Note.—One of the animals in the 1st dose died before the Challenge Dose was given. Hence the total number of animals used is shown as four instead of five as in the other doses.

Conclusion.—The authors reported that about 2.0 μ g of Fraction IA gave 50.0 per cent. protection when tested by their technique. The above results show very close agreement with the results obtained by Karl Mayer. This is an experimental finding of great importance as it helps workers in different places to check up their work.

II. NUTRITIONAL REQUIREMENTS OF *P. PESTIS*—STUDIES ON THE ELUATE FROM CHARCOAL.

(Wagle and Govind).

It has previously been reported that treatment of the acid digest of casein (which we use for the preparation of vaccine) with 'Neuchar' considerably enhances the nutritive value of the hydrolysate for the growth of *P. pestis* (Sokhey, Habbu and Bharucha, 1950, *Bull. World Hlth. Org.*).

It was supposed that the charcoal probably removed some factor or factors inhabiting the growth of *P. pestis* or that such treatment set right an imbalance in the amino acid content of the medium which is responsible for less growth.

In order to extract the inhabiting factor from the charcoal, the 'Neuchar' used for adsorption purposes was boiled with water for 20-30 minutes and filtered under pressure. The eluate so obtained had a pH of 2.3. It was neutralised with the requisite quantity of Sodium Carbonate, and made to pH 7.4 and adjusted to a sodium chloride concentration equivalent to that present in the original broth. Such an eluate contained 3.36 mgms. Nitrogen per ml. and was added to liquid casein hydrolysate in different quantities and the growth of *P. pestis* measured turbidimetrically after 48 hours.

A typical result is given below :—

Tube No.	Contents.		Turbidimetric reading
1	9 cc. Casein hydrolysate +1 cc. eluate	...	67.5
2	9 cc. Casein hydrolysate +0.8 cc. eluate +0.2 cc. water	...	68
3	„ +0.6 cc. eluate +0.4 cc. water	...	66
4	„ +0.4 cc. eluate +0.6 cc. water	...	66
5	„ +0.2 cc. eluate +0.8 cc. water	...	69.5
6	„ +1 cc. water	...	66.5

It is clear from the results that the water eluate of the charcoal does not produce any significant inhibition on the growth of *P. pestis*.

To test whether treatment with 'Neuchar' removed some inhibiting amino acid from the casein digest, two dimensional paper chromatography of the digest before and after treatment with charcoal was done. A reference chromatogram was prepared using synthetic amino acids. The two solvents used were (a) Phenol Solution consisting of 400 ml. pure distilled Phenol, 12.5 ml. of salt solution and 87.5 ml. of distilled water and (b) Butanol-Acetic Acid solution consisting of 250 ml. Butanol to which were added 30 ml. of Glacial Acetic Acid, 13 ml. of salt solution and 237 ml. of distilled water. The salt solution contained 6.3 per cent. Sodium Citrate, 3.7 per cent. Potassium Dihydrogen-Phosphate and 0.5 per cent. Ascorbic Acid.

The solvents were usually allowed to travel 8-10 inches beyond the point of application of the substance. For developing the spots 0.2 per cent. Nihydrin in Butanol saturated with water was used. Qualitatively, there was no difference in the distribution patterns of the amino acids in the casein hydrolysate before and after charcoal treatment.

III. PURIFICATION OF THE SYNERGIC FRACTION FROM CRUDE VEAL-INFUSION PROTEOSE-PEPTONE DIPHTHERIA TOXOID.

(Lahiri).

A study on the distribution of the synergist in different fractions of the crude toxoid indicated that the bulk of the synergist came out in the fraction which dialysed out. This dialysate was further purified by adsorption of the synergist on activated charcoal, and subsequent elution of the adsorbate with 0.2 N NaOH. The purified synergic fraction was almost as powerful as alum as adjuvant to purified diphtheria toxoid.

IV. PRODUCTION OF DIPHTHERIA TOXIN.

(Nimbkar).

Infusion proteose peptone medium has been our routine medium of choice for the large-scale production of diphtheria toxin. However the

toxin production in this medium is very irregular and varies from batch to batch at times giving toxins of 20-30 Lfs. per millilitre and sometimes no toxin at all.

In the preparation of this medium no special attention is paid to the concentration of salts particularly those of iron. Optimum concentration of sugars and the area volume ratio also play a major part in toxin production. Hence an attempt is made to work out the limiting conditions for regular production of toxins in this medium.

For this purpose iron is precipitated out from the medium by precipitation of calcium phosphate in the medium. About 1.5 of CaCl_2 and H_2O is found to be sufficient for reducing the concentration of iron to 0.05 mg. per litre or below. $\alpha\alpha^1$ dipyridyl method is used for estimation of iron. An optimum concentration of iron giving maximum yield of toxin is determined. Similarly 0.5 per cent. of maltose and 0.075 per cent. of glucose is found to aid toxin production. As regards the area volume ratio 200 ml. of medium in 3 litre Fernbach flask is found to give maximum yield of toxin.

With these factors adjusted to their optimum level toxins of fairly high titre 50-60 Lf/ml. are regularly produced and no failure has been observed till today after these modifications were introduced. The value of this method lies not only in the high titre of toxins obtained but also in the consistency with which these results are obtained.

V. PREPARATION OF TRYPSIN INHIBITOR.

(Shanta Rao).

During studies on the action of crystalline proteolytic enzymes on toxins and toxoids, it was necessary to use specific inhibitors to stop the enzyme action at any required stage. Trypsin inhibitor was prepared from "Stoy" and locally available soya bean flour according to the method of Kunitz. Kunitz recommends Nutrisoy-defatted soya bean flakes processed without using heat, since heat inactivates the inhibitor. Not knowing how "Stoy" was prepared, and being unable to prepare very active inhibitor from this source, whole soya bean obtained from Bangalore was powdered, fat removed by ether extraction and the inhibitor extracted according to Kunitz. The preparation, however, was not of practical use as it gave an intense blue colour with phenol reagent thus making it very difficult to study the extent of inhibition. The extraction of inhibitor from soaked beans with seed coat removed was tried and some inhibitor was obtained in this way.

Phaseolus vulgaris (French beans) was then tried as a source of inhibitor. It was found that the water soluble part inhibited tryptic activity whereas the acid soluble part adsorbed on bentonite and eluted by penidine contained an active proteolytic enzyme. In the subsequent experiments where inhibitor was to be used the water extract of *phaselous* preserved with toluene was used.

VI. PURIFICATION OF PEPSIN.

(Rao.)

Highly active pepsin necessary for carrying out the purification of antitoxins by the pepsin digestion method, was prepared as follows:—

Bovine stomachs were freed from fatty tissue and minced. The minced material was put into 4 times its weight of water containing

1 per cent. hydrochloric acid, shaken from time to time for about 3 hours and then kept in cold overnight. Next morning the solution was filtered. Powdered Ammonium Sulphate was dissolved in the filtrate to make a 60 per cent. saturated solution. Pepsin got precipitated and was filtered. The precipitate was pressed between sheets of blotting paper till it was semi-solid and was finally dried in a vacuum desiccator over calcium chloride. Two kilograms of minced stomach yielded about 40 grams of dry precipitate. This material, however, contained considerable amounts of mucilaginous matter and was also rich in blood group substances. Pepsin free from these impurities was prepared by extracting the dry powder with 60 per cent. alcohol in which the impurities were insoluble. Pure pepsin was precipitated from the alcoholic extract by increasing the concentration of alcohol to 80 per cent. The pepsin so prepared was very highly active and was at least 1,000 times as active as the minced stomach. The overall yield was about 50 per cent.

Attempts are being made to obtain crystalline pepsin from the highly active pepsin. Efforts are also directed towards obtaining blood group substances which are a by-product of pepsin preparation, in a highly pure state.

VII. DETOXIFICATION STUDIES IN DIPHTHERIA TOXIN.

(Shrimati Rao and Lahiri).

It has been observed by us in our previous experiments that Diphtheria Toxoid is resistant to digestion with trypsin, whereas the toxin is readily digested. It has also been known that Diphtheria Toxin is heat labile whereas the toxoid is heat resistant. These properties of the toxoid namely resistance to heat, resistance to digestion by trypsin and the non-toxic property were followed during the incubation of Diphtheria Toxin with formalin.

Different amounts of formalin, i.e., 0.2 per cent., 0.4 per cent., 0.6 per cent. and 1 per cent. formalin were added under sterile conditions to Diphtheria Toxin. At different time intervals over a period of three months samples were taken out. The fall in toxicity, the resistance developed to digestion with crystalline trypsin, and to heating, were measured for each of these samples.

In all the mixtures of toxin and formalin most of the toxicity was lost during the first 24 hours, the fall was fairly rapid for the next two days and later on the fall was very gradual. Toxin with 1 per cent. formalin was nontoxic after 12 days, toxin with 0.6 per cent., was nontoxic after 15 days. The toxin with 0.4 per cent. formalin retained 0.0104 per cent. toxicity even after 3 months. The toxin with 0.2 per cent. formalin lost 95 per cent. toxicity in 15 days and after this time the Lfs began falling down and there was a 70 per cent. loss in Lfs after 1½ months and 80 per cent. after 2 months, and the residual toxicity after three months was 0.04 per cent. of the residual Lfs.

The study has led us to the following conclusions:—

1. To detoxicate diphtheria toxin completely a concentration of formalin more than 0.4 per cent. is needed.

2. The resistance to heat denaturation is a very quick and easy way of finding out whether a toxin under incubation is completely detoxicated or not. Even as little as 0.0104 per cent. toxicity can be detected by heating at 70°C for one hour. Completely detoxicated toxin is resistant to heating at 70°C for 1 hour.

3. The resistance to digestion by trypsin runs parallel to heat denaturation.

VIII. PULICIDAL EFFECT OF PYRETHRUM.

(Deoras).

Experiments were conducted with pyrethrum to ascertain its pulicidal value. Pyrethrum extract available at the Institute containing 7.3 per cent. pyrethrins was formulated as a powder, suspension and a smoke. These formulations were tried on a mixed culture of *Xenopsylla cheopis*, *X. astia* and *X. brassiliensis*.

The powder formulation was used at 50 mg. per square foot in a glass jar. Weekly observations on the knock-down effect are recorded in table I. The residual effect gradually dies out by about 4 weeks. This pyrethrum extract was again formulated after six months but there was no knock-down effect. The assay revealed that the pyrethrum content had come down to 0.53 per cent. pyrethrins.

The suspension stood well only for 15 minutes and the knock-down effect was irregular. The smoke formulation showed no knock-down of the fleas.

The work is in progress.

Date of trial.		No. of days after 1st application.	Temperature.	Per cent. Humidity.	Exposure of fleas to powder in Minutes.	Replications.	Knock-down in		Sex ratio per cent. of females to males.	Remarks.
							Treated.	Control.		
7th 1951.	May	7	39°C	55	15	4	75 per cent.	Nil	65 per cent.	20 fleas in each expt.
14th 1951.	May	15	41°C	54	15	4	52 per cent.	Nil	75 per cent.	
21st 1951.	May	21	43°C	51	15	4	30 per cent.	Nil	75 per cent.	
28th 1951.	May	28	41°C	56	15	4	1 per cent.	Nil	...	
4th 1951.	June	...	42°C	65	15	4	Nil	Nil	...	

IX. TYPHOID FEVER IN THE CITY OF BOMBAY.

(Soman and Modi).

Free diagnostic aid in cases of typhoid fever continued in operation during the year 1951, as before. The number of calls attended by the Institute doctor rose again during the months of August, September, October and November, the average monthly figures being as follows:—

Total	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Novr.	December
814	62	44	49	58	77	57	63	125	88	73	69	44

As a routine, blood was collected for triple Widal Test, clot culture, blood culture, Weil-Felix test and agglutination test for Brucellosis. Total and differential counts were done only when practitioners asked for the same. No case of paratyphoid B or paratyphoid C infection was diagnosed either serologically or bacteriologically. The ratio of *B. typhosus* to *B. paratyphosus* A strains isolated appeared to be 7:1 as compared with that of previous year. The following table illustrates the results for the year 1951 :—

Year	Total samples for Widal and culture	Hospital and private	Typhoid scheme	Widal - Culture +	Widal + Culture -	Widal + Culture +	Total Blood Cultures	<i>B. typhosus</i> isolated	<i>B. paratyphosus</i> A
1951	2200	1436	764	141	505	154	4151	487	71

Total number of calls registered was 814, of which 50 were such wherein patient's address was incomplete or the patient refused to give blood. Thus 764 blood samples were collected and examined, of which 314 were diagnosed as cases of enteric, of which 126 were bacteriologically positive. Out of 126 cultures, 16 were *B. paratyphosus* A. As a routine, both blood culture (Hartley's Broth) and clot culture (Glycerine-Peptone-Bile) were done on the same blood sample being equally divided; hence comparative positive results obtained by clot and blood cultures were worked out in 126 cases only. Out of 126 bacteriologically proved cases, both blood and clot cultures were positive in 56, only blood in 29 and only clot in 41.

From Arthur Road Hospital 2,006 samples were received for Weil-Felix test and on routine cultural examination of these, for enteric group of organisms, 231 strains of *B. typhosus* and 32 of *B. paratyphosus* A strains were isolated.

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

Distribution of calls and enteric cases under typhoid scheme.—There were 659 calls from the city and 105 from the suburbs and this distribution according to wards showed the following results:—

	A	B	C	D	E	F	G	Suburbs	Total
Calls received ...	22	4	39	115	76	224	179	105	764
Enteric cases ...	10	...	13	48	35	91	71	46	314

From the data available, it is found that wards D, F and G showed the highest incidence of enteric cases in the city for the year under report, although no account of private and hospital cases has been taken.

X. A PRELIMINARY NOTE ON THE ISOLATION OF INFLUENZA VIRUS.

(Soman).

Soon after my return to the Institute from the Rockefeller Fellowship abroad for study of viruses, there was an opportunity for trying isolation of Influenza Virus because of the prevalence of severe colds during the

months of July and August. The World Health Organisation had already designated the virus laboratory of the Haffkine Institute, Bombay, as one of the W.H.O. Influenza Centres in India and had made a generous gift of equipment costing U.S. \$4,500 to this centre. An adequate and regular supply of fertile eggs was first arranged. Five throat washings collected from patients clinically diagnosed as severe colds or influenza were inoculated amniotically into 13 days old chick-embryos and incubated for 5 days at 37°C. Two strains of Influenza Virus were isolated. On account of the difficulty with which they could be adapted and established in the allantoic cavity, their F/G ratio in the early and late egg passages and the results of HA-inhibition tests on paired sera of the patients from whom they were isolated, these two strains were labelled provisionally as Type B HIB-1/51 and B HIB-2/51, pending final confirmation. The remaining three throat washings were discarded as negative after making three serial blind passages in eggs. Standard strains PR8 and Lee and FM1 had to be kept going in egg passages with a view to collect allantoic fluids as influenza antigens for further work. Potent antisera against strains PR8 and Lee and against two newly isolated strains were prepared in fowls. Soluble antigens from the standard strains and the newly isolated strains for complement fixation are being prepared and the new strains are being further studied.

In 1950, Menon in Coonoor, isolated three Influenza Type A strains and this year Swaminathan in Bombay and Kalra in Poona isolated two similar strains.

The isolation of two Type B strains at this Institute this year is of more than ordinary interest for no Influenza Type B strains have been so far isolated and reported in India from any laboratory. One of the two strains (B HIB-2/51) was sent to Dr. Andrews, W.H.O., Influenza Centre, Mill Hill, London, and he confirmed it to be Influenza Type B, though not identical with Lee strain.

XI. STUDIES ON Q FEVER.

(Soman).

A preliminary report on the complement-fixation tests for the detection of Q fever antibodies in cases of pyrexias of known and unknown origin was published in the Institute's Annual Report for 1949 and no definite serological evidence of such an infection was detected. The Third World Health Assembly realising the increasing importance of Q fever to public health, decided to undertake a survey of the prevalence of this disease in various countries throughout the world. In order to implement this decision for India, where no such disease has been reported so far, Indian Government agreed to the proposed survey being carried out at the Haffkine Institute in Bombay, on account of the availability of necessary facilities and expert personnel. The supplies of Q fever antigen and control sera would be obtained from W.H.O., Geneva. The serological survey necessarily deals with the examination of human sera and sera from domestic animals as cattle, sheep and goats and dogs and small rodents. In this connection, arrangements were made through the Superintendent to obtain two samples of sera from human cases admitted to the pneumonia wards of the Infectious Fever Hospital, Arthur Road, Bombay, the first sample being collected immediately on admission and

the second one during convalescence. Thus 150 human sera were tested by complement-fixation with Q fever antigen and only half a dozen of them have given low titre reactions varying from 16 to 64, which are awaiting further confirmation. In the same way, 52 sheep sera were tested for Q fever antibodies and 2 gave positive results in titre 1:16, 1 in 1:32 and 1 in 1:64. These sera were collected from healthy sheep (Satara breed) which were utilised in the Institute for the preparation of antirabic vaccine. Further work on these lines is in progress and will be reported at a later date.

Having worked at the Rocky-Mountain Laboratory, Hamilton, Montana, U.S.A., where rickettsial diseases are exclusively studied, the cultivation of Q fever rickettsia in embryonated eggs was started at the Institute on my return from the fellowship abroad.

The animal reactions were first studied with the strain of *C. burneti* obtained and then the fertile eggs incubated for 6-7 days were inoculated with 10 per cent. suspension of the infected guinea pig spleen. A few blind passages in eggs were necessary to start with but with 6-7 egg passages, the strain was finally established in the yolk-sacs and the Machiavello stained smears from the yolk-sacs showed large number of rickettsia, *Coxiella burneti*.

XII. INFANTILE DIARRHEAS—BACTERIOLOGICAL ASPECT.

(Shrimati Pavri).

Diarrheas, occurring in infants and children upto two years of age, constitute a well-recognized and much dreaded entity. Despite various attempts made by various workers, the etiology is still obscure, several organisms being incriminated as the causative agents.

In order to get a correct perspective, it was thought better to study several stool samples of normal infants and children upto two years of age, together with the diarrheic samples from the same age group.

Study on normal fecal flora was carried out by dividing the infants in 5 distinct groups according to their age and diet and analysing at least 16 meconial and stool samples from each group. The flora, most commonly encountered, comprised of the following organisms:—

Colon group, i.e., *E. coli* and *Aerobacter aerogenes*, *Lactobacilli*, *Enterococci*, *Micrococci* and aerobic sporebearers.

110 diarrheic stools have been, so far, subjected to a complete bacteriological analysis. The organism, most commonly encountered, nearly in 50 per cent. of the cases, is *Proteus mirabilis*. The other organisms, found in predominance, given in order of frequency, are *Pseudomonas aeruginosa*, *Paracolon*, *Proteus morgani*, *Staphylococci*, *Salmonella* and *Shigella* group of organisms.

The one organism, so far not recognised as a causative agent for infantile diarrheas, is *Proteus mirabilis*, which has been isolated from the maximum number of diarrheic stools as indicated above. Its persistent predominance during the diarrheic stage and its disappearance coinciding with the clinical cure, together with positive slide agglutination with patients' sera (in some cases), all go in support of the theory that this organism appears to be responsible for a large number of diarrheic cases.

LIBERATION OF HISTAMINE BY COBRA (NAIA NAIA) VENOM FROM ISOLATED RAT DIAPHRAGM OF THE RAT.

(Dutta and Anantanarayanan).

Several substances like strychnine, adrenaline, d-tubo-curarine chloride and ammonia are known to set free histamine from skeletal or striated muscle. Study of the cobra venom indicated that it is also pharmacologically capable of releasing histamine from the isolated rat diaphragm. In this experiment a quantitative study of the liberation of histamine by cobra venom from striated muscle of the rat has been carried out.

A portion of the rat diaphragm was placed in 5 ml. oxygenated warm (37° C) Tyrode solution while a similar part of the muscle was immersed in the Tyrode solution containing different concentrations of cobra venom. These solutions were replaced by fresh ones every five minutes. Histamine liberated from the muscle by the venom was biologically assayed on isolated guinea-pig's ileum. The following table shows the amount of histamine liberated by cobra venom. The figures represent mean of five experiments :—

Table.

Concentration of cobra venom per millilitre.	10 μ g.	25 μ g.	50 μ g.	100 μ g.
Histamine released μ g/g. of fresh tissue during 5 minutes.	2.3	3.0	5.2	6.6
Histamine released μ g/g. of fresh tissue during 45 minutes.	9.57	9.26	13.95	17.28

It is evident that cobra venom is a potent liberator of histamine.

XIV. STABILITY OF VITAMIN B₁ IN PRESENCE OF ALCOHOL (15 PER CENT.), SALTS OF IRON AND COPPER.

(Dutta, Anantanarayanan and Mehta).

The stability of thiamine hydrochloride and mononitrate was investigated in aqueous solutions and in different mixtures containing alcohol (15 per cent.) or ferrous sulphate, or ferri et ammonii citras or copper sulphate. These pharmaceutical solutions were prepared and stored at 25-30° C in amber coloured glass bottles for a period of 6 months to a year. The pH of the solutions was adjusted to 5. In simple aqueous solutions or mixtures containing 15 per cent. alcohol, thiamine hydrochloride was found to be more stable than thiamine mononitrate although both of them did not retain their original potencies. Both the salts significantly deteriorated in the course of a few weeks in mixtures containing ferrous sulphate, copper sulphate and ferri et ammonii citras. In the presence of the latter substance, thiamine mononitrate hardly retained any potency at the end of 6 months.

XV. CHEMOTHERAPY OF INTESTINAL INFECTIONS: SYNTHESIS OF N⁴-ACYL DERIVATIVES OF THERAPEUTICALLY ACTIVE SULFONAMIDE COMPOUNDS.

(Shirsat, Patki and Ganapathi).

N⁴-acyl derivatives of the sulphonamide compounds, particularly of sulphanilamide, sulphapyridine and sulphathiazole, have been synthesised by various workers with a hope of developing lipophylic (fat soluble) antitubercular and antileprosy drugs. Very few of the N⁴-acyl derivatives

of sulphadiazine, sulphamerazine, and sulphamethazine in literature. There does not seem to be any report regard to the probable utility of N⁴-acyl derivatives of compounds for the treatment of intestinal infection. The work was undertaken with a view to develop suitable derivatives of amides, which would suffer hydrolysis to free sulphonamido in the intestine, thus building up effective concentration thereof at site of infection without necessarily giving rise to unduly high concentrations of the drug in the circulating blood. To achieve this several acyl derivatives of sulphathiazole, sulphadiazine, sulphamerazine, sulphamethazine have been prepared. The acyl groups include both aliphatic as well as the aromatic compounds. The acyl groups contain the mono-chloro-, dichloro-, trichloroacetyl; formyl-, acetyl-, propionyl-, butyryl-, iso-butyryl-, valeryl-, iso-valeryl-, caproyl-, heptyl-, caprylyl-, pelargonyl-, deconyl-, lauryl-, palmityl-, and stearyl-, radicals. The aromatic group contain substituents such as nitro-, methoxy-, and methyl-, etc., and the arylacyl groups include phenylacetyl etc. Most of the acyl compounds have been prepared by the standard procedure of interaction of the acid chlorides with the requisite sulphonamido compound in presence of pyridine as the condensing agent. In the case of aliphatic derivatives of acyl compounds, derivatives have also been prepared by heating the sulphonamido compound with appropriate acid alone for suitable length of time. In the latter procedure it has been observed by us that the reactivity of the aliphatic acids with the aromatic group decreases with the increase in length of the chain affording lesser and lesser yields of the acylamido compounds, inspite of extended periods of refluxing. Thus while, isovaleric acid could afford some amount of desired acyl compound with all the sulphonamides tried, no amides could however be formed by this method with higher fatty acids.

With a view to develop suitable derivatives of para-aminosalicylic acid for the treatment of tubercular infections of the alimentary canal, several N-acyl derivatives of PAS have also been similarly prepared.

Out of about 135 such derivatives prepared about 100 are being reported for the first time. All the acid chlorides, some of the unavailable aliphatic acids, the basic sulphonamides used in the present work, and intermediates required for the synthesis thereof have been prepared during the course of this work. Preparation of about 50 intermediates so prepared, has been described.

Experiments *in vivo* were carried out by feeding white mice with appropriate amounts of a series of the acyl derivatives of sulphadiazine and the estimation carried out with regard to the amount of the drug absorbed through the alimentary canal, the amount of drug hydrolysed and retained in the intestines, and the concentrations of the free and acylsulphadiazine in the blood have been recorded. Experiments *in vitro* on the hydrolysis of representative members of the acyl compounds by homogenates of the intestinal tissues have been carried out. The effect of chemical constitution on the *in vivo* hydrolysis and absorption of several representative acyl derivatives have afforded some interesting results as to point the way towards the choice of a suitable derivative for the treatment of intestinal infections such as typhoid, cholera and dysentery.

XVI. CHEMOTHERAPY OF INTESTINAL INFECTIONS:

(Shirsat, Sardesai and Ganapathi).

Dr. Mahal has reported the effectiveness of formaldehyde condensation product of sulphanilamide in the reduction of *B. coli* concentrations in the faeces of monkeys (Haffkine Institute, Annual Report, 1942-43, pp. 50). The formaldehyde condensation product of sulphanilamide was found by him to be non-toxic and poorly absorbed from the intestinal tract, in contrast with the parent compound sulphanilamide. Several workers have subsequently investigated the utility of formaldehyde condensation product of sulphathiazole in the treatment of intestinal infections such as *Asiatic cholerae* [Bhatnagar, Fernandes, Ind. J. Med. Res., 38, 279 (1950)].

With a view to further extend the original work of Dr. Mahal, we have prepared several derivatives of sulphathiazole, sulphadiazine, sulphamerazine and sulphamethazine, by condensing a mole of aliphatic aldehydes with the requisite sulphonamide, under a variety of experimental conditions.

A large number of aromatic aldehydes, having various substituents in the benzene ring have also been condensed with the intention of studying the inter-relations between the chemical structure and pharmacological and bacteriological characteristics of this class of compounds. Preliminary work in the direction of studying the splitting of the abovementioned condensation products in the intestinal tract of laboratory animals, has given interesting results. The work is being pursued further with a view to develop a suitable remedy for the treatment of *B. typhosus* and *Vibrio cholerae* and other allied bacterial infections of the intestinal tract.

XVII. STUDIES IN THE CHEMISTRY OF THIAZOLES: SULPHONAMIDO DERIVATIVES OF THIAZOLE.

(Shirsat, Kulkarni and Ganapathi).

It has been disclosed in Indian Patent No. 29093 held in the name of the Director, Haffkine Institute, that the dicondensation product obtained by the interaction of para-acetamino-benzenesulphochloride with 2-amino thiazole in aqueous medium in the presence of acid fixing agents such as sodium bicarbonate or calcium carbonate etc., when heated with one mole of 2-amino thiazole yields two moles of acetyl sulphathiazole, thus affording double the yield of sulphathiazole, otherwise obtainable by the standard procedure.

This work has now been further extended and it is now found out that similar dicondensation products are also obtained when ASC is condensed with 2-amino-5-alkyl thiazoles, where in the alkyl group is methyl, ethyl, propyl and iso-propyl. The dicondensation product in each case has been isolated and characterised. The dicondensation products obtained from 2-amino-4-methylthiazole and 2-amino-4-ethylthiazole could be successfully converted into 2 moles of the respective mono-condensation products by heating with the requisite 2-amino-5-alkylthiazoles however, the dicondensation products obtained by the condensation of ASC with 2-amino-5-propyl-thiazole and 2-amino-5-isopropyl thiazole could not be converted into any detectable yield of the corresponding mono-condensation products by heating with the requisite 2-amino-5-alkyl-thiazoles.

Whether this failure is due to the inherent stability of the dicondensation products obtained in these particular cases or is due to inherent incapacity of the requisite 2-amino-5-alkylthiazoles to bring about the intermediary splitting of the dicondensation products is now under further investigation.

The identity of the above stated mono-condensation products wherever isolated was confirmed by hydrolysis of the same to the corresponding sulphathiazole having an alkyl substituent in the 5 position of the thiazole nucleus and the same were compared with authentic samples of corresponding sulphathiazole derivatives synthesised by the classical procedure.

XVIII. STUDIES IN THE CHEMISTRY OF THIAZOLES: SYNTHESIS OF 5-ACETAMINO-4-NITROTHIAZOLE-2-CARBOXYLIC ACID AND CONVERSION THEREOF TO 5-ACETAMINO-4-NITROTHIAZOLE.

(Kulkarni and Ganapathi).

5-Acetaminothiazole-2-carboxylic acid was nitrated to obtain 5-acetamino-4-nitrothiazole-2-carboxylic acid. This nitro acid was decarboxylated to obtain 5-acetamino-4-nitrothiazole which was identical with the product obtained by direct nitration of 5-acetaminothiazole.

5-Acetaminothiazole-2-carboxylic acid was brominated and it was found that 5-acetamino-4 : 5-dibromothiazole was obtained with the simultaneous elimination of carboxylic acid group. This dibromo compound was identical with the compound obtained by direct bromination of 5-acetaminothiazole.

XIX. STUDIES IN THE CHEMISTRY OF THIAZOLES : SYNTHESIS OF 2-THIOAMIDO-4-HYDROXY-6-METHYLPYRIDINOTHIAZOLE AND OF 2-THIOAMINO-4-METHYL-6-HYDROXYPYRIDINOTHIAZOLE.

(Kulkarni and Ganapathi).

5-Aminothiazole-2-thioamide is condensed with acetoacetic ester in alcohol at low temperature. The condensation product so obtained is cyclised in dioxane to obtain 2-thioamido-4-hydroxy-6-methylpyridinethiazole. If the condensation is done in absence of alcohol at high temperature, the product obtained after cyclisation is 2-thioamido-4-methyl-6-hydroxypyridinethiazole.

XX. STUDIES IN THE CHEMISTRY OF THIAZOLES : SYNTHESIS OF 2-AMIDINO-5-AMINOTHIAZOLES AND DERIVATIVES THEREOF.

(Kulkarni and Ganapathi).

5-Aminothiazole-2-thioamide (Chrysean) is heated with methyl iodide to obtain S-methyl derivatives of chrysean which condenses with aromatic amines such as aniline, p-chloroaniline, p-toluidine etc., to obtain the 2-amidino-5-aminothiazoles.

XXI. A COMPARISON OF "HOWE" ALBUMIN WITH "TRUE" ALBUMIN IN SERUM SAMPLES.

(Bharucha and A. Venkataraman).

The introduction of electrophoretic technique for the fractionation and analyses of serum proteins has clearly shown that the albumin value obtained by the "Howe's" 23 per cent. sodium sulphate fractionation

includes a greater portion of the α -globulin fraction. Since a decrease in serum albumin is always accompanied by a corresponding increase in α -globulin, any such clinical changes will escape detection by the use of the traditional 23 per cent. sodium sulphate for the fractionation of serum proteins. A few published data indicate that the "Howe" albumin values which are still used in many clinical laboratories, conceal both the frequency and magnitude of Hypoalbuminemia.

By chemical fractionation techniques which have been adjusted to electrophoretic standards, it has been worked out that a sodium sulphate concentration of 27 per cent. is required to precipitate all the globulin fractions, leaving only the "true" albumin in solution. Similarly the β and γ globulin values can be obtained by using suitable salt concentrations.

The modified procedure for the determination of albumin and globulin N-by Kjeldahl digestion using 1 : 1 sulphuric acid containing 1 per cent. selenium dioxide, and subsequent nesslerisation, has been found to work very satisfactorily for routine analyses,

With this modification of using 27 per cent. instead of 23 per cent. sodium sulphate it has been possible to show that the "True" albumin values can reach so low a figure as 0.2 and 0.1-g per cent. in some cases of hypoalbuminemia while the "Howe" albumin value would be about 1 g. per cent. The ratio of "True" albumin to globulins would come down to as low as .05 or even .03 in such cases.

XXII. A STUDY OF THE BIURET METHOD OF THE ROUTINE ESTIMATION OF SERUM PROTEINS.

(A. Venkataraman and Bharucha).

Many pathological and normal sera have been analysed by both the Biuret and the modified Kjeldahl methods for their "True" albumin and total globulin contents. Since definite and significant discrepancies have been found in the various pathological serum samples, the Biuret Method which has gained some popularity recently, cannot be substituted in place of the well-known Kjeldahl method of N-determination. However, we are continuing the study to get more data on the discrepancies found and the nature of disease involved. It has been suggested that the ratio of Biuret N (which represents peptide N) to Kjeldahl N might show definite patterns and hence be of diagnostic value.

XXIII. A RAPID METHOD FOR THE DETERMINATION OF SODIUM IN SERUM.

(A. Venkataraman).

The chemical methods for the determination of sodium in serum depend on the precipitation of sodium as a triple acetate with uranium and any divalent metal like zinc, magnesium, etc. The triple acetate is easily soluble in water but insoluble in excess of the reagent, e.g., Uranyl-Zinc-Acetate or in alcohol. A large excess of the reagent is generally used and the washing, especially in microdeterminations, should be thorough. This procedure is not only time-consuming but requires careful and conscientious technique. A great drawback with such a procedure is the

fact that the excess of the reagent, which must be completely removed is washed with a saturated acetone solution of the triple salt itself. Such a step brings in an error of 5 to 10 per cent. when working with standard sodium solutions. This is really serious when we consider that small changes in the value of serum sodium are clinically significant.

Determination of serum sodium by flame photometry using an internal standard is becoming popular. However, as this is an expensive instrument out of reach of many clinical laboratories, a chemical method which would be rapid as well as accurate should be welcome.

We are trying to develop a simpler and reproducible technique to precipitate the sodium and the serum proteins simultaneously by means of zinc or magnesium uranyl acetate reagent containing 80 per cent. v/v alcohol. After complete precipitation and centrifugation the sodium content is calculated from the loss in concentration of the uranium in solution by a colorimetric procedure using potassium ferrocyanide. Our experience so far indicates that a completely satisfactory and rapid method can be worked out with slight modifications of the conditions.

Work on similar lines for the estimation of serum potassium is also in progress.

XXIV. URINARY 17-KETO STEROIDS.

(A. Venkataraman).

The estimation of urinary 17-Keto steroids has become of considerable interest to the clinical endocrinologist. In many laboratories investigations have been carried out in the hope of finding a simple method of estimating these. Nearly every laboratory is using more or less different techniques.

The Zimmermann's method modified in various ways is still frequently used. The colour developed by reacting the dried ether extract of urine in an alkaline m-dinitro benzene solution is fairly stable and reproducible, provided the solvents and reagents are carefully purified and freshly prepared. The presence of varying amounts of non-specific chromogens in the urine extract contributes somewhat towards the higher values obtained by this method. We have found it preferable to apply a blank correction which can range from 5-15 per cent. of the value obtained. Corrections for interfering chromogens have been suggested by various workers by measuring the intensity of the colour at two different wavelengths. A spectro-photometer is recommended for such a study. However, we are trying to use the Photoelectric Colorimeter with the available filters. The simplicity of the conditions as expressed in the formula suggested by Talbot is still to be established.

Side by side we are investigating the colorimetric method of Pincus using antimony trichloride in glacial acetic acid on the dry ether extract. This method has not been widely used on account of the rigorous conditions necessary for reproducible results.

A study of the excretion of the 17-keto steroids in normal individuals of varying age groups and certain pathological conditions is being carried out.

XXV. EXPERIMENTAL HEPATIC NECROSIS.

(Radhakrishna Rao, Krishnan and Govind).

Radhakrishna Rao (Nature, 161, 1948), had pointed out that the differences due to strain variation of the rats might be one of the factors which are responsible for the discrepancies in the production of massive hepatic necrosis. In view of this, it was thought worthwhile to study the influence of topopherol in the production of massive hepatic necrosis in the Haffkine inbred strain of rats and the Wister strain, as outlined in the following experiments. (The Wister strain of rats was obtained through the kind courtesy of Dr. V. R. Khanolkar from the Tata Memorial Hospital, Parel).

(a) *Influence of Tocopherol on the production of Massive Hepatic Necrosis in the Haffkine inbred strain of weanling rats.*—12 weanling rats (21 days old) were distributed evenly, as far as possible, with respect to weight, sex and litter mates into two groups (V and VI) so that each group contained six rats. The two groups of rats with an initial body weight of about 22 gms. were placed on the "yeast protein diet", similar to that used in the above experiment. Each rat was given daily 8 gm. of this diet. One group (group V) received a supplement of alpha-tocopherol (1 mg. per rat per day) and to the second group (group VI) which served as control, no tocopherol was given. The body weights of the animals were recorded twice in a week. A record of the day to day food consumption has been maintained.

It will be of interest to record that one rat from the control group has shown a typical massive necrosis of the liver. The work is in progress.

(b) *The influence of tocopherol on the production of Massive Hepatic Necrosis in the Wister strain of weanling rats.*—10 weanling rats (21 days old) with an initial body weight of 25 gm. were distributed as in the above experiment into two groups (VII and VIII) of 5 each, and were fed 8 gm. of the "yeast diet" per rat per day. One group (group VII) received a supplement of alpha-tocopherol (1 mg. per rat per day) and the other group (group VIII) served as control. The body weight and food consumption have been recorded as outlined above.

The experiment is in progress.

XXVI. VITAMIN E CONTENT OF HUMAN MILK.

(Govind and Radhakrishna Rao).

A study of the normal composition of human milk was deemed necessary during investigations on the ætiology of "Infantile Biliary Cirrhosis". The choline, methionine and cystine content of human milk has already been reported (Annual Report, 1949). The tocopherol content of a few normal samples obtained 3-8 days after delivery are reported here.

Vitamin E was estimated by Emmerie and Engel's iron $\alpha\alpha^1$ bipyridyl reaction. Determinations were done in Unicchem photoelectric colorimeter using Ilford filter No. 404 (green). The milk was heated with trichloroacetic acid to about 3-4 per cent. acid, and filtered after keeping in the cold for 4 hours. The precipitate was saponified by KOH in the

presence of 2-3 ml. of 5 per cent. α^1 Pyrogallol for nearly 4-6 minutes in a water-bath. The unsaponifiable matter was extracted thrice with ether, the ether extract was evaporated on a water-bath under reduced pressure, re-dissolved in Ethyl alcohol-light petroleum mixture and passed through a column of alumina. To suitable aliquots of the filtrate were added 1 ml. of α^1 bipyridyl reagent and 1 ml. of ferric chloride reagent, and the optical densities taken. The results are given below :—

No. of samples analysed.	Vitamin B content in mgms. per 100 ml. milk.	
	Range.	Average.
38	205.1-220	0.618

XXVII. STUDIES ON BLOOD PROTEINS : EVALUATION OF DIFFERENT CLINICAL METHODS FOR ESTIMATION OF TOTAL SERUM PROTEINS.

(Kumari Tara Kundaji).

In the evaluation of different methods for the determination of blood proteins (reported in the Haffkine Institute Annual Report for 1950), the Copper Sulphate method of Phillips et al was found to be most convenient for field investigations. Certain drawbacks were observed in the method—viz., (1) A thin film consisting mainly of red blood cells separates out from the drop of blood as soon as it comes in contact with the Copper Sulphate solution, thereby affecting the specific gravity of the test drops and the standard Copper Sulphate solution; (2) Owing to the partial miscibility of blood and serum and the reference solution of Copper Sulphate, the specific gravities of both are altered.

In view of these shortcomings, several other solutions were tried to replace the Copper Sulphate solution. A mixture of m-xylene (S.G. 0.835) and mono-bromobenzene (S.G. 1.485) was found to be most suitable. A series of tubes containing this mixture with sp. gravities ranging from 1.016 to 1.060 were prepared by mixing the two liquids in suitable proportions and a number of blood and serum samples were tested in it.

TABLE.

Showing the specific gravities obtained for whole blood and serum by using (1) Xylene-Bromobenzene mixture; (2) Copper Sulphate Solution compared with the standard pycnometer values.

Description.	No. of samples.	Method.	S.G. of whole blood.	S.G. of serum.
Normal	90	1. Pycnometer	1.0568	1.0258
		2. Xylene-Bromobenzene method	1.056	1.026
		3. CuSO_4 method	1.054	1.024
Pathological	42	1. Xylene-Bromobenzene method	1.054	1.025
		2. CuSO_4 method	1.051	1.022

It will be clear from the table that the xylene-bromo-benzene method gives values for specific gravity more close to the pycnometer values than the copper sulphate solution. The other main advantages of this modification, are :—

1. Unlike the copper sulphate solution, the drops of serum and blood remain discrete and separate and there is no formation of any films or turbidity. Moreover, owing to the immiscibility of the drops and the reference medium, one single drop can be tested over again.

2. Any moisture that may be absorbed from the test drops can be easily removed by shaking with a small amount of CaCl_2 . Further, the components of the used solutions can be obtained in a pure state by distillation for fresh use.

XXVIII. STUDIES ON BLOOD PROTEINS : AN ANALYSIS OF SERUM PROTEINS AND ITS FRACTIONS IN NORMAL AND IN MALNOURISHED PERSONS.

(Kumari Tara Kundaji).

Samples of normal serum were obtained mostly from the blood donors at the "Blood Bank" in this Institute and the samples from malnourished subjects were obtained from the "Nutrition Clinic" at the J. J. Group of Hospitals.

The total serum proteins were determined by the Kjeldahl procedure and albumin and globulin fractions by "Howe's procedure".

TABLE.

Showing the average total serum proteins and its fractions in normal and in malnourished persons.

	No. of samples	Total protein gm/100 ml.	Albumin gm/100 ml.	Globulin gm/100 ml.	Euglobulin gm/100 ml.	Pseudoglobulin gm/100 ml.
Normals	180	7.23	4.49	2.74	0.56	2.18
Malnourished cases.	42	6.35	3.40	2.95	0.67	2.28

It is obvious from the results that the fall in total proteins in malnourished persons is largely due to the fall in the albumin fraction. In fact the total globulins is only 0.21 gms. per 100 ml. higher than the normal values, whereas the albumin is 1.07 gms/100 lower than the normal value. Of the two globulin fractions, the euglobulin shows a higher proportionate increase in value than the pseudo-globulin, though in both cases the increases are not striking. The tendency to maintain a constant level of globulin in undernutrition may be due to : (1) the greater chances of loss of albumin from the plasma into the tissues, because of the comparatively smaller size of the albumin molecule and (2) the greater need of the body in hypoproteinemic states to maintain the normal pattern of immunologic elements even at the expense of the other protein fractions.

XXIX. SUPPLEMENTARY NUTRITIVE VALUE OF SOME SUBSIDIARY CEREALS.

(Kumari Tara Kundaji).

Recently much attention has been focussed on the subsidiary cereals and it was thought probable that some of these cannot only replace the staple cereals like rice and wheat, but might also increase the nutritive value of diets in general. In the investigation reported here the supplementary nutritive value of some of these cereals has been evaluated under two different conditions.

Experiment I.—When supplemented at 3.25 per cent. protein level to a diet containing a pulse, red gram (dahl arhar) as the other source of protein, by “growth method” using young albino rats.

Experiment II.—When supplemented at 2.8 per cent. protein level to a diet containing rice as the other source of protein, by “maintenance method” using adult rats.

TABLE.

Showing the Biological Values of the different diets in Experiment I.

Diet	Cereal plus pulse			Biological value by 1/p
I	Rice plus red gram	...	...	1.483
II	Wari plus red gram	...	...	0.837
III	Rajgira plus red gram	...	...	1.253
IV	Kodra plus red gram	...	...	1.595

TABLE.

Showing Biological Values and digestibility Coefficient of diets in Experiment II.

	Diet.	Biological Value.		Digestibility Coefficient,
I	Rice	...	89.8	86.9
II	Wari plus rice	...	71.8	90.5
III	Rajgira plus rice	...	86.2	88.3
IV	Kodra plus rice	...	91.5	89.6

It was found that the cereals investigated can be arranged in the following descending order of merit: (1) ‘Kodra’, (2) Rice (3) ‘Rajgira’, (4) ‘Wari’. Analysis of blood and tissue proteins of the rats showed that (1) ‘wari’ was poorest as regards haemoglobin albumin and tissue protein formation; (2) rice and ‘Kodra’ groups showed normal values for albumin, but the globulin level was slightly high in the Kodra group.

In general 'Kodra' is as good as rice when measured by growth and other criteria of assessment of protein value, 'Rajgira' comes a close second whereas 'Wari' is a poor substitute.

XXX. KEEPING QUALITIES OF SEMPLE'S VACCINE.

(Dogra).

This study was completed during the year under report. In all, six random samples of Semple's Vaccine were studied over a period of several months. Sufficient quantity was set aside and stored at 5-8°C for repeated tests. Potency tests were done as early as two weeks after preparation of the vaccine and as long after as sixteen months of storage. The results are set out in the following table.

TABLE.—Showing LD 50 potency values of Semple's Vaccine Stored at 5-8° C.

Period of Storage.		Brew Nos.					
		77/49	140/49	822/49	825/49	833/50	840/50
2 weeks	...	2,512	22,910	19,500	33,110	4,467	11,220
2 months	...	43,650	46,770	1,00,000	61,660	51,290	61,660
4 months	...	11,750	4,467	...	...	...	...
5 months	...	...	...	31,620	31,670	...	...
6 months	...	14,790	14,130	...	...	26,920	27,540
8 months	...	1,950	9,333	14,130	58,880	...	...
10 months	...	562	7,943	57,540	42,660	13,180	10,000
12 months	...	...	724	...	...	...	...
16 months	...	...	...	891	1,380	...	...

From the above data it would appear that any particular brew of vaccine if stored at 5-8°C would retain its potency above 1,000 LD50 level for a minimum period of eight months. It would appear that vaccines of initially lower potency would fall in potency earlier whereas those of a very high initial level may remain above the prescribed level for as long as sixteen months. It may also be noted that all brews improved in potency on storage. The above data would suggest that the expiry period of carbolised antirabic vaccine be extended from six to eight months.

XXXI. PRESERVATION OF SEED VIRUS.

(Dogra).

In the routine large scale manufacture of Semple's vaccine, Rabies seed virus is maintained in rabbit brain. Fortnightly passage is done and the rabbit brain preserved in 50 per cent. glycerine saline and stored

at 5-8° C in a domestic refrigerator for use as and when required. LD50 titre of such seed virus was ascertained with the following results :—

Rabbit No.	Date of test.	Period of storage.		LD50 titre.
48/51	23rd January 1951	...	Fresh	$10^{-5.6}$
148/51	19th February 1951	...	Fresh	$10^{-7.5}$
1041/50	27th February 1951	...	10 weeks	0
2/51	28th February 1951	...	6 weeks	$10^{-4.0}$
87/51	4th April 1951	...	8 weeks	$10^{-4.87}$
*885—386/51	5th April 1951	...	Fresh	$10^{-6.19}$

* Two rabbit brains were pooled.

It would be apparent that the method of preservation of seed virus in vogue was not ideal, in that (a) it was costly and wasteful, (b) the titre varied from brain to brain, (c) the titre deteriorated in course of time and in about 10 weeks, the preserved material showed no evidence of live virus. Even in 6-8 weeks stored samples, the titre was below the required level for propagating Rabies Virus in sheep brain.

Dried Seed Virus.—Attempts were made to lyophilise rabbit brain seed virus with a view to seeing if in the lyophilised form the titre could be maintained at a suitable level for a sufficient length of time. Lyophilisation met with satisfactory results, but this needs further investigation to determine the period over which the titre is maintained. The following data is presented. Further work is in progress. Similar results have been obtained with Rabies Mouse-brain Virus.

Titre of Rabbit Brain Seed Virus after Lyophilisation.

Date.	Rabbit No.	Initial titre.	Titre of dried material.			
			Immediately after lyophilisation.	2 months after.	4 months after.	
5th April 1951	...	385-86/51	$10^{-6.19}$	...	$10^{-5.75}$	$10^{-4.0}$
26th April 1951	...	462/51	$10^{-5.60}$	...	$10^{-4.74}$	$10^{-4.62}$
14th May 1951	...	586-87/51	10^{-7}	$10^{-5.25}$	$10^{-5.12}$	$10^{-6.0}$

XXXII. LYOPHILISED RABIES VACCINE.

(Dogra).

Sample's Vaccine even when prepared over long periods by experienced staff with a standard technique showed variations from brew to brew in potency levels, deteriorates rapidly if stored in the open and loses potency gradually if stored in the cold. It was considered that a lyophilised product, if prepared satisfactorily, would be the product of choice in India where the climate varies so markedly and where distances are great and cold storage facilities limited.

A chloroform killed milled vaccine was prepared and successfully lyophilised. The lyophilised product reconstitutes well and gives a homogeneous emulsion. Preliminary assays have shown it to possess a satisfactory level of potency. Further work is in progress.

XXXIII. PURIFICATION OF RABIES VACCINE.

(Dogra and Shirsat).

Semple's vaccine is a crude vaccine as it consists of dead rabies virus and the entire brain substance of the animal in which the virus is harvested. It has been suggested that the lipid moiety is, possibly, responsible for post treatment paralytic cases. Removal of the lipid material was difficult from Semple's vaccine, but it was easily done by repeated extraction with ether at 29 to 30°C from the lyophilised vaccine. Sterols and allied materials—ether soluble—was removed. The final product reconstituted well, but, when compared with whole vaccine it showed a very low potency. Apparently, the method of extraction with ether adversely affected the immunological proportion of the vaccine.

XXXIV. MOUSE PROTECTION TEST.

(Dogra).

No satisfactory method has been made available, so far, to assay the level and duration of immunity produced in man after antirabic treatment with Semple's vaccine. As a result, the amount of vaccine to be administered after a patient has been bitten by a rabid or suspected rabid animal is not determinable, the extent of danger of a second infection to an already treated individual is undeterminable, and, the whole scheme of vaccine therapy in Rabies has remained on an unscientific basis.

Experience showed that the Paris strain of Rabies Virus could be harvested in mice and lyophilised and used as a fairly constant challenge dose. The breed of white mice available in this Institute could be depended upon for very constant results as these animals have been found to be extremely sensitive to intracranial infection. A large number of patients attend for rabies treatment at the Institute from whom sera could be obtained. For these reasons it was planned to assay the level of immunity in man resulting from Antirabic Treatment by means of a mouse protection test. For purposes of the test, cases were grouped as those who never had any rabies treatment at all and those who received full treatment of Class II and III, 3, 6 and 9 months prior to test. A mouse protection test was devised on lines similar to such tests for similar purposes.

Blood samples were collected and stored in cold. Serum separated and mixed in equal quantities with mouse brain rabies virus with a titre 300 times the dose required to kill 50 per cent. of animals. This was inoculated into 5 mice each receiving 0.03 cc. intracranially. Control animals received the challenge virus, 0.03 cc. each, mixed with equal quantities of deci-normal horse serum. The mixtures were then kept in cold overnight and inoculated into another series of animals. The animals were observed for 21 days after inoculation.

Results with Mouse Protection Test.

Enough experience has been obtained to record that this test gives a qualitative method of determining the immunity produced. A large number of samples will have to be tested before conclusions are drawn, but it may be stated that whereas sera from patients who had not received any rabies treatment showed to possess no antigenic properties, series of patients treated with Class II and III antirabic treatments showed to possess antigenic property even 6-9 months after treatment. This work is in hand.

XXXV. YELLOW FEVER VACCINE AND A MOUSE POTENCY TEST.

(Dogra, Parakh and Vijay Singh).

(a) *Yellow Fever Inoculations.*—An analysis of records of yellow fever inoculations carried out in this Institute over the past 10 years showed that of the 67,740 persons inoculated upto the end of the year under report, in no case did we receive a report of an ill-effect except in one case in which Herpes simplex developed on the arm on which the inoculation was given.

(b) *Keeping Qualities of Yellow Fever Vaccine.*—Repeated mouse potency tests (200) carried out on several batches of the Rockefeller Foundation Yellow Fever Vaccine, stored in the dark at -4°C at this Institute, during the past years, when studied, showed that this aqueous base vaccine kept well for a period of 3-5 years and that the deterioration was gradual.

(c) *Rehydrated Yellow Fever Vaccine and its keeping qualities.*—Three batches were tested for potency titre soon after rehydration and then a portion of it kept in ice and the rest kept at 80°F (room temperature in Bombay) 98°F (the maximum temperature in Bombay). Hourly samples of vaccine were tested to determine the titre of the vaccine. The portion stored at 98°F lost its potency completely very rapidly. The one stored at 80°F lost its potency gradually, but surely, but, the one stored in ice retained its titre at the initial level for 3-4 hours. Further work is in hand.

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PARL, BOMBAY,
June 23, 1952.

P. M. WAGLE,
Director, Haffkine Institute.

