



सत्यमेव जयते

REPORT
OF
THE HAFFKINE INSTITUTE

For the Year 1952

BY

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BOMBAY

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1954

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1952

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 Virus Diseases including Rabies.

Officiated as Director from 1st March 1952 to 4th July 1952 in addition to his duties.

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

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

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

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

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

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 Vaccines in addition to his duties from 1st March 1952 to 4th July 1952.

II. General Review.

In general the activities of the Institute remained the same as in previous years. Production of large quantities of prophylactic vaccines viz., plague, cholera, typhoid and antirabic vaccines, prophylactic and curative antitoxins and toxoids, sulpha drugs and vitamin tablets, parenteral solutions, and diagnostic work for hospitals and practitioners monopolised its resources in funds and personnel. It also served as an analytical laboratory for enforcement of Drugs Act, 1940 and as a provincial bacteriological laboratory for the Bombay State. Despite the pressure of routine duties most of the senior members of the staff carried out research work detailed elsewhere in the report. Some of the research problems bearing on the various activities of the Institute were also carried out with the generous assistance of the Indian Council of Medical Research.

The time of the present staff is fully occupied by laboratory bench work and it does not permit the Institute, the only medical research organization of the province, to play that role in the health organization of the province, which it should, by conducting field and other studies on urgent health problems of the people in close association with the public health department. In 1924 report the then Director strongly stressed the value of such an association and advocated the organization of a mobile unit. The need of such a unit is greater now than ever as is evidenced by the outbreak of so-called "mysterious disease" in Poona district and the need for making a hookworm survey in Kaira district. It is hoped the staff of this Institute will be strengthened to permit of such work being undertaken.

The demand for plague and cholera vaccines was lower than in the previous year. A little less than five million millilitres of plague vaccine and about four million millilitres of cholera vaccine were issued this year. The demand for plague vaccine from various states is definitely on the downward grade as a result of wide scale application of DDT campaign as plague preventive measure in those states and consequent reduction in the incidence of the disease. The lesser demand for cholera vaccine can be attributed partly to lower incidence of cholera infection and partly to the arrangements made by some states to manufacture cholera vaccine in their own laboratories.

Work on casein hydrolysate typhoid vaccine was continued. This vaccine was found to be slightly superior in potency to the agar-made typhoid vaccine and it was hoped, in view of the experience gained with casein hydrolysate plague and cholera vaccines, that this vaccine too would give less reactions in human beings. Inoculations carried out on a small scale in human beings however did not fulfil these expectations and the reaction resulting from the vaccine was found to be of the same intensity as that resulting from agar vaccine.

The work on the nutritional requirements of plague bacillus was continued. The action of the activated animal charcoal "nuchar" in enhancing the growth of *P. pestis* in casein hydrolysate medium was studied by paper chromatography. Chromatograms showed that charcoal did not act by absorbing from casein hydrolysate aminoacids inhibitory to the growth of plague bacillus. Chromatographic study also enabled us to study the amino-acid composition of casein hydrolysate and to determine the various aminoacids utilised by the plague bacillus during its growth in the casein hydrolysate medium. No opportunity occurred to carry out field trials in the value of antibiotics aureomycin, chloromycetin, and terramycin in the treatment of human plague this year.

The demand for the use of antitoxins and sera continued to be in excess of what the Institute could produce. However, the acquisition of the Pimpri Stud Farm on a permanent basis has mitigated, to some extent, the difficulties experienced in meeting the demands from Defence Services and from outside the State. The Pimpri Stud Farm has enabled the Institute to keep a reserve of horses there for preliminary immunization enabling the Department to increase the production of antitoxins and sera by about twenty per cent. over the previous year. With the development of a fullfledged laboratory and provision of necessary technical staff, it would be possible to increase the production still further which in turn would bring in added revenue to the State. The need for exploiting this source of revenue is all the more necessary at the present time than at any other, in view of the fact that another regular source of revenue for the Institute, viz., the vaccines, is steadily going down year by year. This decreasing revenue can be definitely made good by increasing the production of antitoxins in this Institute to double its present capacity.

A new diphtheria prophylactic N.A.F.T. (natural adjuvant factor toxoid) has been manufactured. Field trials with this new prophylactic has shown that it is as antigenic as the alum precipitated toxoid of both local or British manufacture, and at the same time, does not give any reactions in both the adults and children. A great hindrance in the way of active immunization of adults and grown up children against diphtheria viz., the production of unpleasant reactions has, it appears, been removed by the development of this prophylactic.

The Department of Pharmacology had to meet ever increasing demand for testing of drugs and other products and had besides to carry out investigations on problems of fundamental importance pertaining to drugs. The total number of samples examined during the year was 1,524 as against 1,284 in 1951 and 924 in 1950. The progressive increase in the work is due to the tightening up of the Drug Control measures by the various state governments, and also to the increased number of samples sent for examination by manufacturing firms. The percentage of "non-standard" drugs was found to be 33 per cent., as compared to 39 per cent. in 1950, and 38 per cent. in 1951, thereby showing a distinct improvement in the quality of products received during the current year. Under the Drugs Act, 976 products were analysed as compared to 740 in 1951 and 467 in 1950. Of these 598 were examined for the Drugs Controller for the State of Bombay. Amongst the drugs sent by the Drugs Controllers, 37 per cent. did not conform to the claim made on the label. But this percentage represents the percentage of "non-standard" drugs amongst those suspected to be so by the Drugs Controllers and does not represent the percentage obtained by random sampling.

It was pointed out in the last report that Vitamin B₁ was found to be unstable in oral preparations containing especially alcohol, iron and copper. Further work done in the Department has shown that Vitamin B₁ also deteriorates in oral preparations containing Vitamin A acetate, Vitamin C or Yeast extract. In the case of adverse effect of Vitamin C it was found that deterioration of Vitamin B₁ is directly proportional to the concentrations of Ascorbic acid.

The Department of Entomology have undertaken the experiments to evaluate the pulicidal value of pyrethrum formulations. Pyrethrum plants grow in some parts of this country, and the extract of its flowers is known to be a useful insecticide. Experiments to determine its value as a pulicide were primarily undertaken with

as a booster along with pyrethrum extract has however been found to enhance the immediate and residual effects of pyrethrum extract considerably. Experiments were also undertaken to study the conditions necessary for rearing of young snakes under artificial conditions available in the laboratory and for increasing the longevity of adult snakes in the laboratory. For this purpose the snake farm built some years ago has been renovated and brought into use. If success is achieved in rearing young snakes to adult stage and in prolonging the longevity of the adult snakes under laboratory conditions the supply position with regard to venoms of different poisonous snakes which are required in large quantities for the manufacture of antivenene would become considerably easier and more economical from costing point of view.

The demand for antirabic vaccine continued to be on the increase. While the number of persons treated for dogbite were 5,473 in 1950 and 6,489 in 1951, 7,779 persons undertook treatment for the dogbite this year. While no cases of hydrophobia were reported to have occurred amongst those persons treated at the Institute centre, 6 cases of hydrophobia were reported amongst those treated at other outside centres. There is a growing belief that Semple's Vaccine does not give adequate protection in some of those cases of rabid-dog-bites where the bites are on the face and hands. Recent work done in the United States has encouraged the belief that the combined use of vaccine and serum in such cases might give better results. Antirabic serum is therefore being prepared in the Institute and when this is ready, it is proposed to try it out in "face cases" along with vaccine.

The work on the typing of the influenza strains and on the incidence of Q fever in Bombay City has continued. From the influenza cases examined in the laboratory, two strains of type B influenza have been isolated and further work is continuing. From the examination of human sera carried out so far, the incidence of Q fever, although low, has been established in the suburbs of Bombay. Work is continuing. The department also served as an inoculation centre for Yellow Fever and other vaccines for international travellers.

The Department of Chemotherapy continued to manufacture the sulphathiazole tablets and injectables, vitamin tablets and the hydnoceol oil and sulphetrone injectables. The sale of sulphathiazole tablets was somewhat less this year than the previous year as supply to medical practitioners was stopped for the greater part of the year owing to the unsatisfactory conditions of supply of raw materials. From October 1952 however this supply to private medical practitioners was resumed. The sale of vitamin tablets was higher this year than in the previous year. It is felt that this sales can be considerably increased by proper advertisements and distribution through a well-established distributing agency. A total of 2,26,500 ml of hydnoceol oil injectable and 1,70,900 ml of sulphetrone injectable was supplied to various leprosy clinics in the State under instructions from the Surgeon General.

The most significant part of the activity of the Department of Clinical Pathology is that the diagnostic work in the case of infectious diseases, such as enteric fever, dysentery, diphtheria, etc. is carried out entirely free of charge for private medical practitioners. As a result of this, the number of samples examined in this Department have risen from 18,000 to well over 26,000 a year in the last few years. This Department is also serving as a reference laboratory for venereal diseases for the state by issuing standard reagents for the diagnostic serological tests and by helping to check the correctness of these serological tests. This activity is at present confined to Bombay city but it is proposed to extend it to the remaining parts of the State gradually.

The Department of Blood Bank is doing useful work in the supply of dried plasma to hospitals and practitioners. The demand is steady inspite of the fact that new plasma substitutes have appeared in the market. Supply of blood for this purpose is still being received from paid donors and jails, the supply from voluntary donors being totally unsatisfactory.

The demand for parenteral solutions like glucose saline, casein hydrolysate, etc is progressively increasing and since the inception of the section in 1947, has increased almost seven-fold becoming a source of some revenue for the Institute.

Apart from the manufacturing activities and research work carried out by members of the regular staff of the Institute, facilities were provided to some scientific workers to carry out research work under Fellowships offered by the Indian Council of Medical Research, the National Institute of Sciences and by the Bombay Government. Further, facilities were also provided to seven science graduates, on payment of fees, to carry out research work for taking the post-graduate degrees.

The total expenditure for the financial year 1951-52 was Rs. 19,63,748 and the income was Rs. 20,77,243.

III. Department of Vaccines.

During the year under review the demand for Plague and Cholera Vaccine was lower than that of the last year, but that for T. A. B. Vaccine was slightly higher.

4.7 million millilitres of Plague Vaccine, 3.8 million millilitres of Cholera Vaccine and 4,10,754 millilitres of T.A.B. Vaccines were issued in 1952 as against 7 million millilitres, 5.7 million millilitres and 300,000 millilitres of Plague Cholera and T.A.B. Vaccines respectively in the year 1951.

The total quantity of vaccines issued during the year 1952 was about 9 million millilitres.

On the whole the demand for vaccines has continued to show the same downward trend as in last three years due to the application of rigorous preventive measures in the State as well as in the whole of the country.

The demands for Vaccines were received from all parts of the country and for Plague Vaccine from some foreign countries also.

A. PLAGUE VACCINE.

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

Months.					Quantity in millilitres.
January	...	...	...	...	3,56,750
February	...	...	...	...	1,20,520
March	...	...	...	...	1,54,500
April	...	...	...	...	1,28,145
May	...	...	...	...	1,20,740
June	...	...	...	...	1,510
July	...	...	...	...	92,247
August	...	...	...	...	55,201
September	...	...	...	...	5,25,856
October	...	...	...	...	36,460
November	...	...	...	...	6,18,180
December	...	...	...	...	25,42,671

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

Year.					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
1952 (one year)	...	...	...	...	4,752,780
Total ...					257,848,903

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	...	...	...	...	1,65,210
Uttar Pradesh	...	...	...	...	30,09,635
Madhya Pradesh	...	...	...	...	3,56,010
Madhya Bharat	...	...	...	...	10,000
Vindhya Pradesh	...	...	...	...	10,450
Saurashtra	...	...	...	...	350
Rajasthan	...	...	...	...	3,050
Hyderabad (Deccan)	...	...	...	...	2,00,000
Madras State	...	...	...	...	4,800
West Bengal	...	...	...	...	4,37,800
Mysore	...	...	...	...	200
Delhi	...	...	...	...	380
Assam	...	...	...	...	50
Kashmir	...	...	...	...	40
Bhopal	...	...	...	...	10,000
Bihar	...	...	...	...	30,000
Defence Services	...	...	...	...	12,185
Total ...					42,50,160
<i>Foreign Countries.</i>					
Burma	...	...	...	...	5,00,000
Afganistan (through W. H. O.)	...	...	...	...	2,500
Malaya	...	...	...	...	120
Total ...					5,02,620

B. CHOLERA VACCINE.

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

Months.	Quantity in millilitres.
January	27,504
February	29,799
March	5,55,028
April	5,82,089
May	4,29,473
June	3,85,945
July	6,55,009
August	4,77,536
September	1,47,831
October	1,55,146
November	1,77,492
December	1,86,724
Total ...	38,09,576

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	36,900
February	18,996
March	15,961
April	28,806
May	66,050
June	43,804
July	46,929
August	30,475
September	27,734
October	39,920
November	32,692
December	22,487
Total ...	4,10,754

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.

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, 34,000 bottles each of aerobic and anaerobic medium in acid digest broth were prepared. Of these about 2,400 of each were supplied to the Indian Penicillin Committee during the year.

3. The parenteral fluids section shows still further progress during the year. Again this year it was not possible to supply these fluids beyond the Bombay State 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 1952.

Serial No.	Name of Product.	No. of bottles sold.	Amount.
			Rs. a. p.
1	Glucose Saline	38,182	76,264 0 0
2	5 per cent. Glucose Solution	9,562	19,124 0 0
3	Protein Hydrolysate	7,361	14,722 0 0
4	25 per cent. Glucose Solution 100 cc.	4,220	2,110 0 0
5	25 per cent. Glucose Solution 500 cc.	1,218	3,045 0 0
6	Normal Saline	3,267	6,534 0 0
7	Hypertonic Saline	1,772	3,544 0 0
8	Sodium Sulphate, Isotonic	762	1,524 0 0
9	4 per cent. Sodium Citrate	742	1,484 0 0
10	Distilled Water	753	376 8 0
11	50 per cent. Glucose, 100 cc.	103	103 0 0
Total		67,942	1,28,830 8 0

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

1949	...	...	31,718	46,542
1950	...	...	47,111	88,838
1951	...	...	54,892	1,03,232

E. SECTION OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY.

This section mainly examined samples of water for ascertaining their fitness for drinking, sanitary and industrial purposes.

A special reference may be made to the samples of water received from Director of Public Health, Poona. These samples were collected from areas where the so-called "mysterious disease" is prevalent. These samples in addition to the routine chemical and bacteriological examinations were subjected to special bacteriological examination with a view to detect the presence of any pathogenic organisms. But no pathogenic organism could be detected though all these samples showed evidence of faecal contamination. The following table gives the summary of the various examinations carried out during the year 1952 :—

Sample examined.	Nature of examination.	No. of samples examined.
Water	... Fitness for drinking purposes	... 71
Water	... Fitness for Bathing	... 4
Water	... Fitness for sanitary and industrial purposes	... 38
Ice	... Bacteriological examination	... 1

IV. Department of Antitoxins and Sera.

Production of antitoxins and sera reached the highest level of the last several years. Nevertheless, the supply exceeded the production, and hence, the stock was further depleted. While 831,300 cc. of antitoxins and sera were produced, 835,680 cc. were supplied, and yet we had to continue to refuse supplies to parties who were eager to get their requirements of antitoxins and sera from us. However, the facility of housing a reserve of horses at Pimpri has shown its effect in considerable increase in the production. When a full-fledged laboratory and the required technical staff is provided to make the Pimpri Farm an additional unit of the department of antitoxins and sera, the production must still further improve, and we might be able to meet all the demands that are repeatedly made upon us for supply.

Field trials with the new diphtheria prophylactic N.A.F.T. (natural adjuvant factor toxoid) in comparison with the A.P.T. (alum precipitated toxoid) of local and British manufacture respectively, has shown further evidence of freedom from reactions following administration of the prophylactic into both adults and children. Moreover, though the prophylactic did not cause any reactions, it was almost as highly antigenic as the alum precipitated toxoid. One of the greatest hindrance in the way of active immunization of adults and grown up children against diphtheria viz., the production of unpleasant reactions in the immunized, has, it appears, been removed.

The anti-human rabbit serum floccule was tried further in the field for the control of other allergic states than the horse serum reactions. The results have so far been encouraging. It has proven its safety beyond any reasonable doubt, and its efficacy in the treatment of allergic state has been shown in several cases. Further field trials are continuing.

The Tiselius electrophoretic apparatus which was obtained from Adam & Hilger

compared with antitoxins. Samples of sera from kala-azar patients were obtained from the Calcutta School of Tropical Medicine and their electrophoretic patterns were determined. Bovine lens protein was characterized. This new line of investigation is being explored thoroughly.

One of the members of the department Dr. D. C. Lahiri was invited by the world Health Organization to participate, firstly in the Conference of Heads of Laboratories producing Diphtheria and Pertussis Vaccines, held in Dubrovnik, Yugoslavia from 13 to 18 October 1952, and secondly in the sixth session of the Expert Committee on Biological Standardization, held in Geneva from 20 to 25 October 1952. He attended both of these meetings.

1. *Horses*.—The total number of horses used in the production of antitoxins and sera during the year was 170. Besides these, there were 76 horses undergoing preliminary immunization at the end of the year. One horse was reserved for production of normal horse sera. The horses were grouped as follows :

Antitoxine.				Number of horses used.
1. Tetanus Antitoxin	...	...	...	88
2. Diphtheria Antitoxin	...	...	...	39
3. Antivenene	...	...	...	36
4. Welchii Antitoxin	...	...	...	7
5. Preliminary immunization	...	...	...	76
6. Normal Horse Serum	...	...	...	1
				247

2. *Preparation of Toxins and Toxoids*.—During the year diphtheria, tetanus and welchii toxins were prepared for hyperimmunization of horses. The reserve stocks of the other toxins sufficed during the year. Due to the larger number of horses under immunization the quantities of diphtheria and tetanus toxins prepared were more than 50% over the production of the previous year. A part of the diphtheria and tetanus toxins were converted into toxoids for immunization of horses. A portion of these toxoids were reserved for human use.

Types of Toxins and Toxoids.				Quantity in litres.
1. Tetanus	...	...	...	526
2. Diphtheria	...	...	...	210
3. Welchii	...	...	...	39
4. Septique	...	...	...	Nil.
5. Oedematiens	...	...	...	Nil.
6. Shiga	...	...	...	Nil.
7. Tetanus toxin converted to toxoid	...	...	...	45
8. Diphtheria toxin converted to toxoid	..			174

3. *Preparation of Antitoxins and Sera.*—During the year a total volume of 7,542 litres of horse blood was handled from which 5,122 litres of plasma were recovered. The volume of processed antitoxins and sera made available for human use amounted to 831,000 ml. which is the highest in the history of the department and is an increase of about 27 percent over production in 1951. The quantity of each type of sera was as follows:

No.	Name.	Amount of concentrated serum in ml.
-----	-------	-------------------------------------

I. CONCENTRATED SERA.

1. Tetanus Antitoxin	...	...	...	465,300
2. Diphtheria Antitoxin	...	...	...	174,800
3. Antivenene	...	...	...	148,400
4. Anti-gas Gangrene (Welchii)	...	...	...	42,800
Total				8,31,300

II. NATURAL SERA.

1. Normal Horse Serum	...	...	...	11,200
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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 71,090 ml.

5. *Supply.*—The sale value of the products during the year, was Rs. 5,46,721 which is about 25 percent increase over the previous year. In addition to the list of items shown in the table below, 12,420 ml. of Natural Horse Serum was supplied free to local hospitals. The following quantities of antitoxins, sera, and toxoids were issued during the year under review.

1. <i>Tetanus Antitoxin</i> —			
(a) Prophylactic 3,000 I.U. in 2 ml.	...	...	3,63,598
(b) Curative 10,000 I.U. in 5 ml.	...	...	7,980
(c) Curative 20,000 I.U. in 10 ml.	...	...	1,08,360
(d) Highly concentrated 20,000 I.U. in 4 ml.	...	...	56,524
2. <i>Diphtheria Antitoxin</i> —			
(a) Prophylactic 1,000 I.U. in 2 ml.	...	...	4,258
(b) Curative 5,000 I.U. in 5 ml.	...	...	3,010
(c) Curative 10,000 I.U. in 10 ml.	...	...	1,32,300
(d) Highly concentrated 10,000 I.U. in 5 ml.	...	...	5,695
3. <i>Gas-Gangrene Antitoxin</i> —			
(a) 3,000 I.U. Welchii Antitoxin in 10 ml.	...	...	72,520
(b) 9,000 I.U. Welchii, 4,500 I.U. Septique and 9,000 I.U. Oedematiens Antitoxins, in a volume not exceeding 15 ml., highly concentrated and purified	...	...	5,025

No.	Name.	Amount of concentrated serum in ml.
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II. NATURAL SERA—contd.

6.	<i>Anti-dysentery Serum—</i>	
(a)	Natural, 20 ml.	1,260
(b)	Concentrated 4,000 I.U. in 5 ml.	210
7.	<i>Tetanus Toxoid—</i>	
(a)	10 ml.	170
(b)	5 ml.	710
(c)	1 ml.	856
8.	<i>Diphtheria N.A.F.T. Toxoid—</i>	
(a)	5 ml.	185
(b)	1 ml.	184
9.	<i>Lyophilised Schick Test Toxin for 25 tests</i>	<i>Nil.</i>
10.	<i>Normal Horse Serum 10 ml.</i>	2,450
Total : 835,680 ml. of antitoxins and sera, and 2,105 ml. of toxoids.		

6. *Other activities of the Department.*—The department undertook to test the potency of 18 samples of tetanus antitoxin and 5 samples of diphtheria antitoxin for the drug standardization section of the Haffkine Institute.

A staff member of Serum and Vaccine Laboratory, Alabang, Muntinlupa, Rizal, Philippines, Dr. Genaro SyChangco, came to us as a Fellow of the World Health Organization. He was given a thorough training in the production of toxins and toxoids, in the immunization of horses for the production of antitoxins, in the concentration of antitoxins, and in the standardization of antitoxins and toxoids during the four months he was in the Institute.

V. Department of Entomology.

During the year, technical assistance was given to health authorities of different states of the Indian Union in connection with the information on Plague work with particular reference to Fleas, Rats, rat traps, Pulicides and Rodenticides. Several enquiries from institutions and private bodies in connection with insect pests in storage, insects of medical importance, scorpions, earthworms, jellyfish, lizards, and sharks were attended to.

Two courses of training in Plague and Plague preventive measures were conducted for the benefit of Epidemic Medical Officers and qualified Sanitary Inspectors deputed by the Public Health Department of the Government and Local Bodies, when 38 Officers deputed by the Government of Bombay, Madhya Pradesh and Saurashtra attended the Courses.

Nearly 2,500 persons belonging to various institutions and private parties came on Thursdays to witness the snake venom demonstration and gather information about the activities of the Institute. Among the above were about 1,600 students from 11 Medical, 5 Agriculture, 4 Veterinary, 11 Science and Arts Colleges, and 25 Secondary and Primary Schools.

Breeding of fleas *X. cheopis* and *X. astia* for epidemiological studies were continued. Life history studies of these, during different seasons and the morphological studies on a comparative basis were also continued.

Experiments to evaluate the pulicidal value of Pyrethrum formulations were continued. The introduction of Piperonyl butoxide as a booster was found to enhance the immediate and residual effect of Pyrethrum on fleas. Some of these observations are incorporated in a summary form in Part II of this report.

Preliminary experiments were done to study the rodenticidal value of some of the modern anticoagulant rat poisons, and the rat virus. Laboratory and field experiments with "Dethmor" (Warfarin) have shown that all the important species of Bombay rats are killed by it, and the rats do not develop bait-shyness. But the cost works out to be comparatively more as the baiting is to be required continued for atleast 15 days before any appreciable results are seen.

Experiments with the Danish Ratin virus showed that it is not effective against any of the common Bombay rats, i.e. *R. rattus*, *R. norvegicus* and *Gunomys kok*.

Flea population of the trapped rats, particularly *R. rattus* and *Gunomys kok* were studied with particular reference to the sex ratio of the parasite and the host. Experiments are also being conducted to study the breeding habits in captivity and burrowing habits of *R. rattus* and *Gunomys kok*.

36 flea-pool experiments were also done to test the presence or absence of plague infection in them.

A. SNAKES.

Fifty snake specimens which were sent by hospitals and private bodies with history of bites were identified. Twentyfive enquiries and requests for the information and articles on snakes were attended to. During the year 939 snakes comprising of 33 Cobras, 88 Russell's vipers, 31 Kraits, 797 Saw-scaled vipers were milked for venom collection. The quantity of different venoms collected and supplied during the year under report was as follows :

Cobra venom (dry weight)		Russell's viper venom (dry weight)		Krait venom (dry weight)		Echis venom (dry weight).	
Collected	Issued	Collected	Issued	Collected	Issued	Collected	Issued.
gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
64.467	15.89	102.80	25.18	2.23	2.05	7.36	2.46

The demand for snake venoms from foreign research laboratories continued to come and were met with.

The alterations in the structure of the open-air snake farm were completed and it is now being used for keeping snakes.

The production of antivenene against the four common poisonous snakes of India depends upon the availability of venom at this Institute, but during recent years it had become increasingly difficult to get enough supply of snakes for this purpose. A scheme to study the feeding and breeding habits of the snakes with a view to make the supply question less difficult was sanctioned by the Indian Council of Medical

TABLE No. III.—Showing the number and percentage of different

Species.	January.		February.		March.		April.		May.		June.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus rufescens</i> ..	24,817	24.4	19,608	20.9	19,793	21.0	17,585	19.0	16,546	19.1	16,227	19.6
<i>Rattus norvegicus</i> ..	16,718	16.8	16,919	18.1	17,224	18.3	17,552	19.1	15,917	18.4	14,490	17.5
<i>Gunomys kok</i> ..	35,366	35.5	36,318	38.8	37,848	40.1	38,420	41.8	35,606	41.1	33,702	40.8
<i>Bandicota malabarica</i> ..	1,097	1.1	966	1.0	747	0.8	707	0.8	715	0.8	987	1.2
<i>Mus dubius</i> ..	10,814	10.8	8,109	8.7	6,705	7.1	5,676	6.2	5,605	6.5	5,024	6.1
<i>Suncus caeruleus</i> ..	11,361	11.4	11,671	12.5	11,948	12.7	12,072	13.1	12,262	14.1	12,196	14.8
Total ..	99,673	100.0	98,691	100.0	94,265	100.0	91,962	100.0	86,641	100.0	82,626	100.0

TABLE No. IV.—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> ..	345	45.8	286	44.7	211	34.5	236	39.4	263	41.6	212	40.9
<i>Rattus norvegicus</i> ..	156	20.7	115	18.0	131	21.4	137	22.8	140	22.1	105	20.2
<i>Gunomys kok</i> ..	185	24.6	197	30.8	202	33.1	188	31.3	136	21.5	125	24.1
<i>Bandicota malabarica</i> ..	...	...	...	...	...	...	5	0.8	11	1.7	10	1.9
<i>Mus dubius</i> ..	12	1.6	...	...	...	...	2	0.3	2	0.3	1	0.2
<i>Suncus caeruleus</i> ..	2	0.3	...	...	1	0.2	4	0.7	2	0.3	3	0.6
<i>Putrid</i> ..	53	7.0	42	6.5	66	10.8	28	4.7	79	12.5	63	12.1
Total ..	753	100.0	640	100.0	611	100.0	600	100.0	633	100.0	518	100.0

species of rats trapped alive by Municipal Corporation during 1952

July.		August.		September.		October.		November.		December.		Whole year.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
16,181	21·8	20,563	24·2	23,362	23·1	25,791	25·1	25,576	25·6	26,969	25·1	2,54,468	22·6
15,523	18·6	16,008	18·8	17,230	17·1	16,266	15·8	17,595	17·6	18,452	17·2	1,99,894	17·7
31,558	37·9	30,063	35·4	38,301	38·0	37,708	36·8	35,014	35·1	39,408	36·7	4,29,312	38·1
826	1·0	712	0·8	786	0·8	1,004	1·0	1,085	1·1	1,370	1·3	11,002	1·0
5,113	6·1	5,986	7·1	8,147	8·1	8,438	8·3	7,700	7·7	8,011	7·4	85,328	7·6
12,203	14·6	11,669	13·7	13,015	12·9	13,360	13·0	12,849	12·9	13,184	12·3	1,47,780	13·0
83,404	100·0	85,001	100·0	1,00,841	100·0	1,02,567	100·0	99,819	100·0	1,07,394	100·0	11,27,784	100·0

found dead in the City of Bombay (including the Suburbs) during 1952.

July.		August.		September.		October.		November.		December.		Whole Year.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
221	47·9	200	53·5	271	57·4	132	38·1	126	54·8	34	33·0	2,537	44·2
61	13·2	62	16·6	55	11·7	50	14·4	37	16·1	15·6	16	1,065	18·6
126	27·3	43	11·5	89	18·9	113	32·7	41	17·8	14	13·6	1,459	25·4
3	0·7	1	0·3	3	0·6	3	0·9	...	..	...	..	36	0·6
6]	1·3	2	0·5	1	0·2	1	0·3	...	..	2	2·0	29	0·5
...	..	2	0·5	...	..	2	0·6	1	0·4	...	..	17	0·3
44	9·6	64	17·1	53	11·2	45	13·0	25	10·9	37	35·9	599	10·4

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

TABLE No. 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> ..	478	61·0	403	49·0	472	55·2	543	66·3	365	55·6	369	59·1
<i>Rattus norvegicus</i> ..	84	10·7	108	13·2	75	8·8	69	8·4	88	13·4	91	14·6
<i>Gunomys kok</i> ..	93	11·8	153	18·6	128	15·0	105	12·8	101	15·4	68	10·9
<i>Bandicota malabarica</i> ..	6	0·8	6	0·7	2	0·2	1	0·1	6	0·9	·7	1·1
<i>Mus dubius</i> ..	65	8·3	72	8·8	143	16·7	72	8·8	70	10·6	44	7·1
<i>Suncus caeruleus</i> ..	58	7·4	80	9·7	35	4·1	29	3·6	27	4·1	45	7·2
Total ..	784	100·0	822	100·0	855	100·0	819	100·0	657	100·0	624	100·0

BOMBAY PORT TRUST AREA.

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 Health Commissioner.

of rats caught alive from the Bombay Port Trust area in 1952.

July.		August.		September.		October.		November.		December.		Whole Year.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
321	55.3	362	60.0	368	63.3	299	50.7	295	42.3	383	53.8	4,658	56.0
60	10.3	82	13.6	87	15.0	87	14.7	102	14.6	60	8.4	993	11.9
115	19.8	84	13.7	56	9.6	130	22.0	130	18.7	97	13.6	1,260	15.1
5	0.9	...	..	...	..	4	0.7	13	1.9	6	0.9	56	0.7
38	6.5	30	5.0	41	7.1	34	5.8	112	16.1	119	16.7	840	10.1
42	7.2	46	7.7	29	5.0	36	6.1	45	6.4	47	6.6	519	6.2
581	100.0	604	100.0	581	100.0	590	100.0	697	100.0	712	100.0	8,326	100.0

TABLE No. VI.—*Showing the different localities in the Bombay Province from which smears and rats were received for examination during 1952.*

Name of Town.	No. of smears.	No. of rats.
Nadiad	2	
Chikodi	4	
Thana	1	1
Shrirampur (Ahmednagar)		1
Ahmednagar	4	
Karad	2	
Islampura	2	
Total	15	2

TABLE N . VII.—*Bombay City Rat supplied to different institutions during the year 1952.*

Name of the Institution.	Insecticide Officer.	Wilson College.	Ramnarain Ruia College.	Natural History section Museum.	G. S. Medical college.
No. of rats supplied.	30	290	130	4	27

Name of the Institution.	Ruparel College.	Siddharth College.	Topiwalla National Medical College.	Khalsa College.	Institute of Science.	Jai Hind College.
No. of rats supplied.	10	14	12	14	184	47

Total No. of rats supplied :—762.

C. EXAMINATION OF RATS FROM STEAMERS AND OTHER SOURCES.

During the year 1952, 2 dead rats and 15 smears of dead rats were received from different towns of the Bombay Province. None of them was found to be plague infected. In the same year, 530 killed rats were received from the Health Officer of the Port of Bombay and of these 497 belonged to the species *Rattus rattus rufescens* 4 *Rattus norvegicus*, 2 *Bandicota malabarica* and 27 *Mus dubius*. None of them was found plague infected.

During the same year, 21 rats were received from the City of Bombay through other agencies or private persons. None of them was found to be plague infected.

VI. Department of Clinical Pathology.

The Department of Clinical Pathology continued to (1) conduct bacteriological serological, microscopic and biological tests on clinical material received from Government hospitals and dispensaries as well as from private practitioners and institutions; (2) prepare, stock and issue diagnostic reagents and stains; (3) carry out histological examination and report on pathological tissues; (4) prepare autogenous vaccines, and conduct special tests such as the Rideal Walker Test; and (5) train graduates and others for laboratory duties; and afford facilities for post-graduate research students. The Histology Section was accommodated in the department itself instead of the Department of Nutrition and Experimental Pathology, the latter having been separated from the Institute for administrative purposes.

A. BACTERIOLOGICAL DIAGNOSTIC SECTION.

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

The Government of Bombay *Typhoid Scheme* was continued for the fifth year and found very popular, and useful. 864 calls were attended to. Blood samples were collected for blood culture and Widal tests. Some practitioners required additional laboratory tests which were also done. All positive cases were reported to the Executive Health Officer, Bombay Municipality, Bombay, to enable him to take the necessary preventive measure at the earliest opportunity. In this examination of the 834 blood samples for culture and Widal, 294, i.e. 35 per cent were positive. This low percentage is due to some patients being on Chloromycetin treatment. It is found that it is futile to examine blood for blood culture and Widal test for suspected early typhoid case if the patient is on Chloromycetin treatment for the previous 3 days.

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

(b) *Results of blood culture and Widal test.*

Year.	Total samples.	Hospital samples.	Typhoid Scheme samples.	Widal— Culture.	Widal+ Culture.	Widal+ Culture.	Total Cultures done.	B.typho- sus isolated.	B. Para A isolated.
				+	—	+			
1950 ..	2747	1935	812	167	524	229	2747	339	88
1951 ..	4151	3387	764	141	505	154	4151	487	71
1952 ..	4189	3355	834	161	646	151	4189	366	57

This Department worked as a reference laboratory on behalf of world Health Organization for venereal diseases control programme. Small quantities of V.D.R.L. and Meinicke's antigens were received and a few representative samples of tested sera from 1,160 samples were checked. Of these, there was agreement with our results to the extent of 92 per cent. This work is in progress. Arrangements are underway to extend this checking work to all venereal clinics in the State of Bombay.

The number of examinations carried out during the year 1950, 1951 and 1952 are shown in the following table for purposes of comparison. It will be observed that there was an increase in the number of examinations done during the year under review as in the previous year.

	1950.	1951.	1952.
<i>I. Serological Examinations—</i>			
(1) Wassermann Test	4,977	4,536	3,661
(2) Kahn Test	1,683	2,589	2,627
(3) Triple Widal Test	2,354	2,167	2,687
(4) Agglutination Test for—			
(a) Typhus	2,796	4,206	3,142
(b) Brucellosis	2,369	2,186	2,811
(c) Leptospirosis	56	37	64
(d) Paul Bunnell Test		10	19
<i>II. Bacteriological and Biological Tests—</i>			
(1) Blood Culture for Enteric Group	2,747	4,151	4,189
(2) Urine Culture for Pathogenic Organisms	172	113	67
(3) Throat Swabs for Diphtheria	120	189	238
(4) Stools and Rectal Swabs for Culture	1,855	809	1,152
(5) Sputum and Body Fluids for A. F. B.	521	671	733
(6) C. S. F. for Culture	295	414	461
(7) Material from Suspected Plague cases	10	11	14
(8) Material from Medico-Legal cases	14	27	33

III. General Microscopic Examinations—

(1) Blood Films for M. P. and Filariasis	..	..	676	409	54
(2) Blood Cell Count	..	..	872	1,363	1,621
(3) Bone Marrow	..	..		22	6
(4) Semen for Fertility	..	..	56	50	56
(5) Material for Smear Examination (Presence of Pathogenic Organisms like G. C. etc.)			105	56	51
(6) Stool-Routine Test	..	..	958	1,057	1,068
(7) Urine-Routine Test	..	..	665	978	890
IV. Pregnancy Test	..	..	318	386	523
V. Rideal Walker Test	..	..	54	30	47
Total			23,673	26,407	26,214

Autogenous Vaccines :

Use of autogenous vaccines is still in vogue amongst the practitioners in Bombay. Cases of chronic inflammatory conditions such as sinusitis, follicular pharyngitis and gingivitis, etc., etc., were indications for such vaccines. The number of courses issued is shown below :—

	1950	1951	1952
Courses of autogenous vaccines supplied	57	56	55

2 Diagnostic Reagents.

(i) *Supply of Diagnostic Reagents.*—The work of production and supply of various reagents for diagnostic purposes to laboratories all over the State of Bombay and outside the State was continued. The demands for these reagents are progressively increasing as may be seen from the following yearly supplies for the last three years.

Reagents.	Quantity supplied in ml.		
	1950.	1951.	1952.
(1) Killed suspensions for Widal and Weil-Felix Tests	1,01,910	1,19,920	1,60,485
(2) Blood Grouping Sera	4,225	5,719	7,170
(3) High Titre Sera & A Biceptor	390	729	756
(4) Kahn Antigen	1,437	3,964	5,825

(ii) *Supply of Stains*.—In addition to diagnostic reagents, the supply of various biological stains was made and a total of 106,430 ml of different strains were issued to different state and private laboratories.

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

B. HISTOPATHOLOGICAL SECTION.

Pathological Specimens.—During the year under review, 1,685 pathological specimens received from different hospitals in the State of Bombay and a few from outside the State were examined. These included brain specimens from suspected rabid animals, neoplasms, gynaecological conditions, tuberculosis of different organs and some rare and interesting pathological specimens. In addition, 444 specimens from experimental animals were examined to study the morbid histological changes. These were mostly from different departments of the Institute as well as different research enquiries conducted at the Institute. In all, 2,129 specimens were subjected to histological examination.

The classification of the specimens of tissues received from hospitals in the City of Bombay and outstations during the years 1950, 1951 and 1952 is set out in the following table :—

Classification of pathological specimens received for examination

Classification.	1950.	1951.	1952.
(1) Tissues found to be normal	55	143	284
(2) Tissues unfit for examination	88	107	136
(3) Brain tissue for Negri Bodies	93	52	79
(4) Miscellaneous Pathological conditions	504	609	884
(5) Tuberculosis	47	74	123
(6) Non-malignant Neoplasms	73	74	65
(7) Malignant Tumours	84	92	114
Total ..	944	1,151	1,685

It is evident that there has been a marked increase in the demands for Histopathological Examinations.

Some of the important and interesting types of pathological conditions encountered are grouped and tabulated.

I. NEOPLASMS.

(a) *Malignant :*

(1) Carcinoma Primary—

Adenocarcinoma	...	...	...	...	14
Anaplastic Carcinoma	...	...	...	...	3
Basal Cell Carcinoma	...	...	...	...	2
Epidermoid Carcinoma	...	...	...	...	34
Diffuse Carcinoma	...	...	...	...	1
Medullary Carcinoma	...	...	...	...	4
Scirrhus Carcinoma	..	...	...	..	1
Carcinoma simplex	...	...	...	...	19

(2) Carcinoma Secondary ... 8

(3) Sarcoma—

Fibrosarcoma	...	...	...	...	5
Angiosarcoma	...	...	...	...	1
Liposarcoma	...	...	...	...	1
Round cell sarcoma	...	...	...	...	2
Melanotic sarcoma	...	...	...	...	3
Mixed cell sarcoma	...	...	...	...	5

(4) Malignant Lymphoma—

Hodgkin's Disease	...	...	...	...	1
Lymphosarcoma	...	...	...	...	6

(5) Seminoma ... 3

(6) Hæmangio endothelioma ... 1

(b) *Non-Malignant Neoplasms :*

Condyloma	...	...	...	...	1
Condroma	...	...	...	...	1
Colloid Goitre	...	...	...	...	2
Diffuse Goitre	...	...	...	...	1
Cystadenoma	...	...	...	...	1
Duct Papilloma	...	...	...	...	3
Fibroadenoma	...	...	...	...	8
Fibrolipoma	...	...	...	...	3
Fibromyoma	...	...	...	...	14
Fibroma	...	...	...	...	9
Fibroma Cellular	...	...	...	...	1
Granuloma	...	...	...	...	3
Hæmangioma	...	...	...	...	1
Lymphangioma	...	...	...	...	1
Lipoma	...	...	...	...	2

I. NEOPLASMS—*contd.*(b) *Non-Malignant Neoplasms—contd.*

Pseudomucinous cystadenoma	...	...	...	...	2
Rhinoscleroma	...	...	...	...	1
Rhinospodiosis	...	...	...	...	1

II. TUBERCULOSIS IN DIFFERENT ORGANS.

Breast	...	...	...	...	2
Female Genital Tract	...	...	...	...	57
Lymphnodes	...	...	...	...	27
Intestinal Tract	...	...	...	...	15
Other Tissues	...	...	...	...	20
Tuberculoma	...	...	...	...	1

Interesting specimens received during the year were :

(1) No. 1122 of 12th December 1951—Endometrial biopsy was found to be Tuberculous Endometritis. The patient was given 12 gms. of Streptomycin and P.A.S. and the subsequent biopsies showed as :—

No. 250 of 4th March 1952 Report :—Endometrium Normal.

(2) No. 422 of 1952—Biopsy Deltoid, Biceps Muscular and Gastronemous showed muscular Dystrophy.

(3) No. 1359 of 1952—Biopsy Tongue showed T.B. Tongue.

Training and Research Scholars : (a) Miss K. M. Pavri, Government of Bombay Scholar continued her studies on "Infantile Diarrhoea".

(b) Shri H. L. Rajguru was trained in laboratory methods including morbid histological technique from 10th April 1952 to 10th October 1952.

Practically every senior member of the staff conducted research alone or in collaboration with others. Detailed description of research work done is given in Part II of this Report.

VII. Department of Pharmacology.

The present report describes the work done during 1952 and this period has seen an activity which is greater than any of the previous year. The department had to meet the ever increasing demand for testing of drugs and other products and side by side it had to investigate on problems of fundamental importance pertaining to drugs. Owing to the heavy pressure of the routine work, not much time could be devoted for research, never the less, some progress have been made in this direction.

The total number of samples examined during the period under review was 1,524 as against 1,284 tested during 1951 which showed an increase to the extent of about 20 per cent. over the last year. Below is the comparative figures of the number of samples tested during the last three years—

	1950	1951	1952
Number of samples tested	924	1,284	1,524

The progressive increase in our work is the direct result of the tightening up of the drug control measures by the various State Governments and also partly due to the increased number of samples tested on behalf of manufacturing concerns

A variety of substances were received for testing. It covered a wide range of pharmacopoeial and non-pharmacopoeial preparations such as alkaloids, glucosides, essential and edible oils, synthetic substances, hormones, vitamins, antibiotics, antitoxins, vaccines, parenteral solutions, surgical dressings, patent preparations, insecticides, laboratory glasswares, etc. Samples were received from many parts of India, viz., East Punjab, Uttar Pradesh, Delhi, Madhya Pradesh, West Bengal, Bombay and Cochin.

Out of 1,524 samples examined 517 were not of standard quality. The percentage of the 'non-standard' drugs decreased from 39 in 1950, 38 in 1951 to 33 in 1952 thereby showing a distinct improvement in the quality of the products received during the current year.

Samples examined under the Drugs Act

The total number of samples examined under the Drugs Act was 976 out of which 598 were from the State of Bombay, 194 from East Punjab, 93 from Uttar Pradesh, 55 from Delhi and 36 from Madhya Pradesh. About 37 per cent. of these samples did not conform to the claim made on the label. It should be remembered that drugs inspectors who were responsible for collecting these samples were in many instances specially looking for misbranded drugs and therefore truly speaking the percentage of 'failed' samples does not represent random sampling. It is hoped that with more stringent drug control measures and with greater efforts on the part of the manufacturers to sell only standard quality products, there would be a steady decline of 'non-standard' drugs.

Schedule C and C1 drugs

Owing to the limited facilities available for testing of 'biological and special products' and 'other special products' in this country, most of the work in this connection was referred to this laboratory both by the States' Drugs Inspectors and manufacturing concerns. The biological and special products tested during the year constituted about 64 per cent of the total samples as against 53 per cent. in 1951. This category products require considerable time for their examination.

Examination of drugs by chemical means

Owing to the brevity of the space it would not be possible to give detailed list of all the products tested here as they are so numerous and diverse in nature or to mention the reasons for non-compliance to the specific standard of each individual drug of 'non-standard' quality. However, some salient features are presented for the benefit of those who are interested in this line.

Dispensing of the liquid Extract of Ergot remained a problem with the manufacturer as this preparation is known to be very unstable in our country. Out of the 13 samples tested, 12 did not conform to the pharmacopoeial standard. Almost all of them had deteriorated in potency.

Seven samples of Liniment of Turpentine were tested, out of which only one conformed to B.P. The volatile oil and camphor content were higher in all cases.

Only 4 samples of Tincture of Iodine were examined but in 3 the iodine and potassium iodide content were either higher or lower than the B.P. specifications.

Out of the 34 samples of Tincture Zingiberis, 20 did not conform to B.P. standard. In a number of samples the strength of the alcohol was not properly adjusted.

One of the chief difficulties which the manufacturer experienced was the elimination of suspended matter in parenteral solutions. The nature of the suspended matter encountered were foreign particles specially bits of glass, fibrous matter and in rare instances the drug itself. Quite a large number of injectable preparations had to be rejected on this account which otherwise would have been regarded as products of quality. Several reasons have been advanced for the presence of such suspended matter, such as difficulty in cleaning the containers thoroughly, quality of the glass containers and improper caps and the absence of a satisfactory mechanical device to detect the presence of suspended matter.

Twenty-two samples of sulfonamides were tested; out of which 6 were found to be not of standard quality. Whereas the content of the active ingredients were always found to be correct, some of the samples 'failed' in other requirements, such as 'Time of disintegration' and 'Uniformity of weight of tablets'. In one case the tablets labelled as 'Sulfadiazine' were found to be 'Sulfathiazole'.

As many as 24 surgical dressings which included cotton wools, lints, bandages and gauzes were examined physically, chemically and also bacteriologically (in the case of those marked 'sterile'). Not a single sample, which was stated to be sterile proved to be so on bacteriological examination. Evidently, the way in which the cotton, lints and gauzes were packed did not prove to be a sufficient safeguard against the entry of bacteria. Chemically also, quite a number of samples of cotton and lint did not comply with the tests for 'absorbency' and 'water soluble extractives'. In a number of instances, the cloth used for preparing bandages and gauze or lint did not comply with the specification laid down for 'Average number of threads per inch' or 'superficial area per ounce'.

Examination of drugs by biochemical and microbiological methods.

This category included (1) most of the vitamins, viz., Anuerine hydrochloride, Riboflavin, Ascorbic acid, Folic acid, Niacin, pantothenic acid, Vitamin A and E, (2) Insulins, and (3) enzymatic preparations. Out of 270 multivitamin preparations tested, as many as 139 were found not to conform to the declared potency. In most of these samples, vitamin B₁ was found to be less. Testing of multivitamin oral preparations presented the most difficult problem as most of them contained ingredients numbering anything between 6 and 15. It has already been pointed out in our last report that vitamin B₁ is most unstable in oral multivitamin preparations, specially in the presence of some inorganic ions. Based on this findings some of the manufacturing firms have altered the formula of their products, whereas others have preferred to add vitamin B₁ in excess amount in order to counteract the progressive loss of potency. Vitamin C and Folic acid contents were also less in a number of these preparations. Anuerine hydrochloride in injectable form was found to be fairly stable. Vitamin B₂ seems to be one of the most stable vitamins.

As many as 20 preparations containing vitamin A were analysed. These included cod liver oil, shark liver oil, vitamin A concentrate and a few so called water soluble vitamin A. In 9 samples the vitamin content did not agree with the declared potency. In most of them it had deteriorated.

Eight samples of insulin were tested by rabbit blood sugar method. In three, the potency was less than the declared amount. One of them was 'time-expired' sample and the other two were locally made and sent here for preliminary testing.

A number of enzymatic preparations were tested. They mostly contained pepsin, papain or diastase. It was rather significant to note that the pepsin contents of Glycerine of Pepsin were invariably found to be lower than the stated amount in majority of the cases.

Microbiological assay of vitamins is one of the important tools in modern analytical techniques. In certain highly coloured preparations containing Riboflavin and Nicotinic acid or its amides, specially those mixed with minerals and other ingredients, considerable difficulty was experienced in analysing these vitamins by fluorimetric or colorimetric methods. Reliable results have been obtained in such cases when microbiological assay was adopted.

Biological assay and standardisation.

Amongst the drugs examined under this category, the following may be mentioned—Digitalis, Strophanthus, Adrenaline, Pituitary, Blood plasma, Hormones, Casein Hydrolysate, Glucose Saline, Redistilled water, Histamine, organic arsenicals and antimonials.

Most of the samples of Tincture of Digitalis received under the Drugs Act were found to be of standard quality. Some of the manufacturing firms had sent us regularly concentrated Tincture of Digitalis for standardization. Thirty three tinctures were tested during 1952 as against 25 during the preceding year.

The number of samples tested for the absence of pyrogenic substances were 273 out of which only a few samples produced pyrexia in rabbits and had to be rejected.

Besides the chemical examination, the organometallic compounds like Sulpharsphenamine, Neoarsphenamine, antimonials were tested for absence of undue toxicity. As a routine, toxicity tests for casein hydrolysate were performed on white mice.

Examination of drugs by bacteriological and serological methods.

The products which were tested bacteriologically were antibiotics, injectables, surgical dressings, catguts, antitoxins and vaccines.

One hundred and two samples of antibiotics (Penicillin and its salts—77 Dihydrostreptomycin 15 Terramycin 8, and Chloromycetin 2) were assayed. All these samples were of foreign-make. Loss of potency was encountered only in 14 samples of Penicillin. Amongst the samples that were of standard quality there were quite a number of Procaine Penicillin and Dihydrostreptomycin whose dates of expiry had passed and still they had retained their full potency.

Under the Drugs Act, 16 samples of antitoxins were tested, the majority of them were Tetanus. Only in two, the potency was less than the declared amount.

VIII. Department of Chemotherapy.

As a result of the exhaustive efforts employed to increase the overall percentage yield of Sulphathiazole, by variations in the conditions and the working techniques of the various stages involved in the manufacture, the department was able within its limited resources to attain an output of 4860 lbs. of Sulphathiazole during the year under review, and furthermore due to improved processes that have been worked out and are at present in progress, and the systematic adjustment of the schedule of operations, the department is in a position to double its output if provided with a few additional items of machinery.

The Sulphathiazole manufactured during the year was processed into tablets and injectables and supplied to all the Government and Municipal hospitals. Supply to medical practitioners which was stopped for some time owing to shortage of raw materials was resumed during October 1952 owing to improvement in supply position of the same.

Extensive investigations in the manufacture of the other Sulpha drugs was also undertaken, with special emphasis on the large scale production of Sulphamerazine. A detailed procedure for its manufacture has been successfully worked out and standardised and its application on a pilot plant scale is at present in operation.

The preparation of the injectables 'Sulphetrone' and 'Hydnocarpus Oil'—the drugs used in the treatment of leprosy was continued as usual, and a constant supply to the leprosy clinics was maintained. A total of 2,26,500 ml. of Hydnocarpus Oil and 1,70,900 ml. of Sulphetrone was manufactured during the year.

About 5,250 lbs. of antiseptic solution "Pemon" was prepared and supplied. Phenyl mercuric nitrate which is used in the preparation of Pemon, and as a preservative in the vaccines manufactured at the Institute was also prepared.

Furthermore the researches conducted in the chemotherapy of malaria, tuberculosis and bacterial infections was continued during the year; a very large number of new compounds were synthesised and the results of the anti-malarial and anti-bacterial activities of the same by in vitro and in vivo tests are eagerly awaited. Summaries of certain portions of the work undertaken has been reported in the research section of the report.

Vitamin Tablet Section.

The regular manufacture of the various kinds of vitamin tablets was continued during the year under review. In addition a good number of Sulphathiazole tablets were also manufactured.

The sale of vitamin tablets through an approved agency is yet under consideration. In spite of the fact that the tablets are sold only at the Institute counter the daily sales are rising up considerably. Also orders from outside the Bombay city are increasing. The need, therefore, for an approved agency for the sale of these tablets is acutely felt.

As regards the Government and Municipal Hospitals and dispensaries, it is observed that their needs are increasing. Many private and charitable hospitals, mills, companies etc., are using these tablets for free distribution to their staff through their dispensaries. A large section of the public will get the benefit of this section only when the tablets are marketed and properly advertised, to make it known to the common man.

A comparative table below shows the number of tablets of various kinds manufactured during the past years and in the year under review :—

Manufacture of Tablets.

Kind of Tablet.	Production during				
	1948.	1949.	1950.	1951.	1952.
1. Vitamin A-D ...	7,05,470	17,82,620	26,63,450	33,36,000	34,41,000
		*40,00,000	30,06,000†	99,000**	95,000*
2. Vitamin B-C ...	7,35,030	17,91,010	34,30,250	44,60,600	46,80,000
		40,00,000	30,06,000†		2,000*
3. Vitamin B-C No. 3			34,560	2,11,550	2,94,500
4. Vitamin B-1 ...	1,73,845	2,44,150	1,29,500	1,98,500	1,70,700
5. Vitamin B-2 ...	36,900	69,290	57,400	79,600	21,750
6. Vitamin C ...	1,99,600	4,11,800	2,96,900	2,87,100	2,34,000
7. Acid Nicotinic ...	16,320	1,65,450	1,14,800	1,29,500	1,30,500
8. Sulphathiazole ...	1,08,000	8,46,655	11,13,000	27,82,000	18,56,000
Total ...	19,75,365	133,10,975	138,52,860	115,83,850	109,25,450

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

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

**Prepared as special tablets of special composition.

IX. Department of Biochemistry.

During the year under review the total number of analyses carried out increased from 4,264 in 1951 to 6,552 this year. Clinical materials were received from the hospitals and private practitioners in the City and to a very small extent from outside districts. The following table gives a summary of the various examinations carried out during 1952 as compared to the previous two years :—

	1950.	1951.	1952.
Blood ...	1,684	2,170	2,637
Urine ...	194	179	149
C.S.F. and body fluids ...	638	1,236	1,764
Stool samples ...	6	3	9
Milk* ...	92	471	1,596
B.M.R. ...	27	31	20
G.T.T. ...	49	57	163
Gastric ...	30	20	45
Kidney function ...	16	59	138
Urea concentration ...	26	34	24
Ketosteroids ...		6	7

The sudden increase of milk samples since November 1951 has added considerably to the pressure of routine work in the Department. It is also necessary to point out that the number of samples for blood proteins, blood urea, cholesterol, serum sodium and potassium, glucose tolerance, gastric, kidney function and Lange's gold chloride tests have increased almost two-fold since the previous year. These estimations are not only time-consuming but some of them require in addition, careful manipulations and control.

The Seamen's Medical Examination Scheme made a special arrangement to send some seamen for glucose tolerance test. These are cases found temporarily unfit to go on sea due to glycosuria. In the course of the year, 83 seamen were examined for glucose tolerance. Due to heavy work in the department it was not possible to take more than 2-3 cases per week. It is observed that the seamen so far examined are either Muslims or Goanese Christians with a history of long term on the sea with somewhat set or typical nutritional habits. The data being collected might yield interesting results for study.

Work on the estimation of 17-ketosteroids is being continued with a view to develop a simplified and reproducible method for routine work and to gather data on normal values for different age-groups.

An excellent modified procedure has been developed for the estimation of serum sodium using 0.1 cc. of the sample.

For the estimation of potassium in serum, the formation of the potassium-cobalt-nitrite complex has been the basis of almost all recent clinical methods barring that of flame photometry. The insoluble cobaltinitrite salt is washed with a wash solution to eliminate other reacting substances and the cobalt present is allowed to reduce.

Folin's phenol reagent in the presence of glycine, as described by Abdul-Fadl. Only 0.2 cc. of serum sample is required for this micro procedure.

Two technicians were given training in the department for six months.

A brief summary of the research work carried out in the department is reported in Section II.

X. Department Virus Diseases including Rabies.

The department continued to (a) afford anti-rabic treatment to patients in northern part of the island of Bombay; (b) prepare anti-rabic vaccine for issue to anti-rabic treatment centres; (c) assist in the diagnosis of rabies in animals; (d) hold stocks of and conduct yellow fever inoculations with the assistance of Yellow Fever Diagnostic Unit sanctioned by the Government of India; (f) conduct research in specific virus diseases like Influenza, Q Fever and Rabies.

Inter-regional Rabies Conference and Seminar sponsored by the World Health Organization was held at Coonoor in July 1952 and Dr. D. W. Soman was permitted to attend the Conference under a W. H. O. Fellowship. The W. H. O. Rabies Consultant, visited this Institute after the Conference and worked in the department for ten days on the preparation of avianized vaccine for canine prophylaxis. Facilities were also provided to two W. H. O. fellows—Dr. Hell from Austria and Dr. Thambiayah from Ceylon to visit and study the preparation of avianized vaccine in the department during Dr. Komarov's visit.

(a) *Antirabic Treatment Centres at this Institute*: During the year under report, 7779 persons applied for antirabic treatment, of these 7092 completed the full course of treatment and 687 were "advice cases". In cases where there was a doubt about the condition of the dog inflicting the bite and the dog was available for observation, patients were given two injections and were advised to keep the dog under observation for 10 days. At the end of the period of observation, if the dog was healthy, no further treatment was given and the case was classified as 'advice case'.

Study of statistics regarding patients who applied for and submitted to treatment, presents interesting data, which is shown below in Table I:—

TABLE I.—*Details of treatment carried out at the treatment centre of this Institute since 1949-52.*

Details.	1949	1950	1951	1952
No. of persons who applied for treatment ...	5,413	5,473	6,489	7,779
Advice cases	775	539	624	687
Fully treated	4,620	4,934	5,865	7,092
Discontinued treatment	18	nil	nil	nil
Reported cases of hydrophobia from cases treated at the Institute centre.	nil	nil	nil	nil

(b) *Preparation and supply of Antirabic Vaccine*.—Five per cent phenolised sheep-brain vaccine (Sample) was prepared as usual and issued to various antirabic centres recognised by the Institute for supply within and outside the Bombay State. Total number of such centres increased from 139 to 147 during the year, out of which 73 were in Bombay State. The vaccine was also supplied to the Veterinary Surgeons throughout the State and outside for treatment of animals. 3470 ml. of 20 per cent canine vaccine was also prepared and supplied for prophylactic use in dogs.

The production and supply of vaccine in the form of treatments, the number of treatment centres, the number of animals used for production have all gone up considerably during the last five years as seen from the following Table II. The production and supply of antirabic vaccine in millilitres month by month is shown in Table III:—

TABLE II.

	1948	1949	1950	1951	1952
Number of treatments sent to all centres.	10,641	11,044	11,468	13,432	17,604
Number of treatments sent to Veterinary Surgeons.	565	675	606	646	827
Reported cases of hydrophobia...	nil	nil	1	nil	6*

TABLE III.—*Production of antirabic vaccine and supplies in ml. during 1952 month by month.*

Month.	Number of ml. vaccine manufactured.	Number of ml. vaccine issued.
January	1,63,500	1,51,204
February	1,78,540	1,82,734
March	1,68,660	1,75,902
April	1,87,980	1,72,259
May	1,02,980	2,03,621
June	1,70,960	1,66,166
July	2,01,460	1,63,756
August	1,52,920	1,53,980
September	1,82,460	1,73,500
October	2,38,620	1,94,254
November	2,07,880	1,89,349
December	2,83,780	1,78,949
	23,39,740	21,05,674

A total of 23,39,740 ml. of antirabic vaccine was manufactured at this Institute and 21,05,674 ml. was supplied to various antirabic centres during the year. The total number of treatments issued during the year was 17,604 and when classified according to three classes showed the following distribution :—

Class I	513 treatments.
Class II	11,408 treatments.
Class III	5,683 treatments.
	17,604 treatments.

Following eight centres for antirabic treatment were added during the year, taking the total number to 147. They are shown below along with the dates of their opening :—

- (1) The Municipal Dispensary, Karad, Bombay, 16th February 1952.
- (2) The Municipal Hospital, Surat, Bombay, 25th March 1952.
- (3) The Janapad Hospital, Harda, M. P., 14th May 1952.
- (4) The Municipal Dispensary, Kapadwanj, Bombay, 13th June 1952.
- (5) The W. F. Pierce Memorial Hospital, Wai, Bombay, 14th October 1952.

- (6) The Civil Hospital, Dwarka, Bombay 16th October 1952.
 (7) The Municipal Hospital, Dohad, Bombay, 15th November 1952.
 (8) The Municipal Dispensary, Akalkot, Bombay, 3rd November 1952.

(c) *Diagnosis of Rabies in Animals*.—Brains of suspected rabid animals are sent to the department for the diagnosis of rabies and are received from Bombay City and the districts. When they are sent from up-country or districts, they are usually preserved in formalin. They are, therefore, only suitable for examination by histological methods. If they are received in glycerine the diagnosis is usually made by examination of impression smears, and by histological methods if necessary. When no negri bodies are demonstrated by these methods they are subjected to a biological test in mice. Out of 80 brains received 53 were sent in glycerine and 27 in formalin.

TABLE IV.—*Number of brains received for diagnosis of rabies from Bombay City and out-stations during the year 1952.*

				Total	Positive.	Negative.	Unfit for examina- tion.
Bombay City	..	..	...	50	26	24	0
Outstations	..	..	...	30	12	14	4
Grand Total				80	38	38	4

TABLE V.—*Statement showing the classification of different animals with the result of their brain examination for rabies during 1952.*

Animal.				Total	Positive.	Negative.	Unfit for examina- tion.
Dog	..	..	..	71	37	31	3
Cow	..	..	..	2	..	2	..
Bullock	..	..	..	1	..	1	..
Buffalo	..	..	..	1	..	1	..
Horse	..	..	..	2	..	1	1
Goat	..	..	..	1	1	..	..
Rabbit	..	..	..	1	..	1	..

(d) *Yellow Fever Vaccine and Inoculation.*—The yellow fever inoculation work, and the testing of potency of the yellow fever vaccine stocked here on behalf of the Government of India, was carried out by the Yellow Fever Diagnostic Unit at this Institute. Several batches of the Rockefeller Foundation Yellow Fever Vaccine were again tested for potency. The total number of potency tests performed during this year was 20.

Yellow Fever inoculation figures during 1952 are shown in Table VI. The persons inoculated were air or sea passengers from Bombay proceeding to, or passing through yellow fever epidemic areas. Figures for 1950 and 1951 are given for purposes of comparison :—

TABLE VI.

Month.	Number of persons inoculated.		
	1950	1951	1952
January	402	667	753
February	876	705	570
March	686	529	462
April	856	883	719
May	715	3250	779
June	569	571	859
July	607	250	685
August	966	567	706
September	639	595	663
October	725	667	785
November	681	694	650
December	539	817	722
Total	8,261	10,195	8,353

The Yellow fever vaccine stored at this institute was distributed during the year to the following Yellow Fever Inoculation Centres :—

Centre.	Quantity of vaccine supplied in doses	
	1951.	1952.
1. The Chief Medical Officer, Porbunder	440	480
2. The Port Health Officer, Jamnagar	500	400
3. The Director of Sanitary and Health Services, Ceylon, Colombo.	Nil.	2,000
4. The Director General of Medical and Health Services, Goa.	600	560
5. The Provincial Public Health Laboratory, New Delhi	2,080	1,280
6. The King Institute, Guindy, Madras	800	2,000
7. The All India Institute of Hygiene and Public Health Calcutta.	4,000	6,000
8. The Air-port Health Officer, Santa Cruz, Bombay ..	2,460	760

(i) *Yellow Fever Vaccine and Rehydrated Yellow Fever Vaccine: its Keeping Qualities.*—As a result of repeated potency tests, it was observed that most of the batches of vaccine received here in 1946, had retained the requisite amount of potency after 6 years of cold-storage at a temperature of 0 — 4°C. Some of them were found to have kept up their full potency so far.

Six more experiments were put up to determine the potency of rehydrated yellow fever vaccine kept in ice. The initial titre of the vaccine, and the titre after 4 hours' storage in ice were worked out. As will be seen from the results shown in Table VII, the fall of potency at the end of 4 hours' cold storage, ranged between $10^{-0.5}$ and $10^{-0.9}$, most of the batches showing a regular fall of $10^{-0.8}$.

TABLE VII.

Batch No.						Initial Titre.	Titre after 4 hours storage in ice.
1841	..	..	..	..	..	$10^{-4.78}$	$10^{-4.0}$
1847	..	..	..	..	..	$10^{-4.25}$	$10^{-3.5}$
1857	..	..	..	..	..	$10^{-3.20}$	$10^{-2.40}$
1906	..	..	..	..	..	$10^{-3.20}$	$10^{-2.40}$
1850	..	..	..	..	..	$10^{-3.50}$	$10^{-2.70}$
1931	..	..	..	..	..	$10^{-4.0}$	$10^{-3.50}$
1927	..	..	..	..	..	$10^{-3.50}$	$10^{-1.80}$
1793	..	..	..	..	..	$10^{-3.25}$	$10^{-3.0}$

8,353 yellow fever inoculations were carried out during the year. No cases of post-vaccinal reactions were reported.

XI. Blood Bank Department.

During the year under report the department continued its normal activities. The demand for dried plasma continued to be the same inspite of the fact that new plasma substitutes have been introduced in the market.

The work of the Mobile Blood collecting Unit was held in abeyance for 4 months as a result of an unfortunate accident to the Medical Officer and the ambulance while on a visit to Nasik for blood collection.

The work of examination for blood grouping was transferred to this department from the Clinical Pathology Department and is being carried on here.

There has been a considerable increase in the demand for the high titre blood grouping sera.

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

2,588 blood donors were screened by CuSo₄ specific gravity method for their fitness to give blood, out of which 1,173 donors were rejected giving a rejection rate of 45·3 per cent.

2,536 blood samples were tested for Kahn tests of which 499 samples were positive, i.e., 22·5 per cent.

10,800 ml of blood with high titred hæmagglutinin was supplied to the Diagnostic Department for preparation of blood grouping sera.

2,472 samples of blood were grouped according to ABO system. The following is their percentage :—

AB	—	210	(8·5%)	B	—	783	(31·7%)
A	—	631	(25·6%)	O	—	848	(35·2%)

TABLE 1.—*Statement showing the collection of blood plasma yielded and distribution of dry plasma in the year 1952.*

Centre.	Type of donor.	No. of donors bled.	Total No. of donors bled.
Haffkine Institute	.. Paid Voluntary Jail donors	1,415 9 442	1,866
Indian Red Cross	.. Paid Voluntary	848 5	853
Other Hospitals	.. Voluntary	82	82
Grand Total			2,801

TABLE 2.—*Showing the yield of blood plasma.*

Product.	Quantity in litres.	Bottles.
Blood	674·6	
Liquid plasma	407·75	
Dried plasma	375·0	1,500

TABLE 3.—*Showing the distribution of plasma.*

Nature.						Quantity in litres. Bottles.	
Sold	...	...	...	...	...	319.75	1,279
Free	...	...	...	...	...	101.25	405
Total						421.00	1,684

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

The number of cases referred for investigation	...	200
The total number of individuals tested	...	341
The number of persons found Rh negative	...	13
Percentage of Rh negative persons	...	3.8%

A case of Anti-‘P’ agglutinin was encountered during the course of study. The case is being reported elsewhere.

XII. Training of Health Personnel.

Serial No.	Name.	Designation and Address.	Department in which trained.	Duration of training.
1	Shri V. B. Kulkarni, G.B.V.C.	Research Assistant, Bombay Veterinary College, Bombay.	Media Department ...	9th January 1952 to 14th April 1952.
2	Shri H. L. Rajguru, B.Sc., Dip. Tech.	Camp Satara-North ...	Department of Biochemistry and Department of Clinical Pathology.	10th January 1952 to 10th October 1952.
3	Dr. Genaro Sy. Chango.	World Health Organization Fellow from Phillipines.	Department of Anti-toxins and Sera.	29th January 1952 to 30th May 1952.
4	Shri D. H. Tejani, M.B.B.S., F.C.P.S., B.M.S. (II).	Blood Transfusion Officer, J. J. Group of Hospitals, Bombay.	Blood Bank Department.	19th February 1952 to 29th February 1952.
5	Dr. B. N. Kharas	Assistant Medical Officer, Municipal Hospital, Surat.	Department of Virus Diseases including Rabies.	7th May 1952 to 13th May 1952.
6	Dr. B. S. Yajnik	Assistant Director, Provincial Hygiene Institute, U. P. Lucknow	Vaccine Department and Clinical Pathology Department.	26th June 1952 to 2nd July 1952.

XIII. Critics Club.

During the year there were 47 weekly meetings when various departments gave talks or read abstracts of current literature in 'their' subject. The following special talks were held during the year:—

- (1) Dr. A. Komarov, on
"Avianised Rabies Vaccine".
- (2) Dr. C. J. Hackett, on
"Newer Aspects of Yaws Control as a Means to the Development of Rural Health Services."
- (3) Dr. Karl Meyer, on
"Plague Vaccine".
- (4) Dr. P. M. Wagle, on
"My Observations of Scientific Laboratories in U.S.A."
- (5) Dr. D. C. Lahiri, on
"The Proceedings of Dubrovnik Conference on Diphtheria and Whooping Cough Vaccines and Meeting of the Expert Committee on Biological Standardization at Geneva."
- (6) Dr. M. V. Radhakrishna Rao, on
"W. H. O. Nutrition Experts' meeting at Fajara".

In addition, two meetings of Teaching Pathologists of Bombay were held in the Institute during the year. At the first meeting the following papers were read held on 1st March 1952:—

- (i) Detoxification of Diphtheria Toxin by Shrimati Shanta Rao.
- (ii) A Case of Anti-P-agglutinin in Blood by Dr. K. V. Kulkarni.
- (iii) Bacteriological Aspect in Infantile Diarrhoea, by Kumari K. M. Pavri.

At the second meeting held on 29th August 1952 the papers read were as follows:

- (i) The Isolation of Enteric Group of Organisms from Contaminated Blood Samples by Kurmari Z. K. Bharucha.
- (ii) Antigen-Antibody reactions in Semi-solid Media By S. S. Rao and M. E. Kulkarni.
- (iii) Isolation of M. tuberculosis—Concentration techniques compound by H. K. Mhatre.

XIV. Miscellaneous.

Apart from the researches carried out by the members of the staff certain scientific enquiries sponsored by Indian Council of Medical Research were also conducted during the year 1952. They were 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 I/c Department of Chemotherapy.
- 3 Enquiry on the Role of Nutritional Factors in Hepatic Cirrhosis. Under Dr. M. V. Radhakrishna Rao, Assistant Director I/C Nutrition Department.
- 4 Enquiry on the Study of Indian Poisonous Snakes. Under Dr. P. J. Deoras, Assistant Director, I/c Department of Entomology.

In addition to above-mentioned, the scientific enquiries conducted here, the following research fellowships and scholarships were attached to this Institute during the year 1952. Particulars of the sponsors, the subject of fellowship and the candidate working under them are as under:—

Serial No.	Sponsors.	Subject of Fellowship.	Candidate working on the Fellowship.
1	National Institute of Sciences of India, New Delhi.	Investigation of Biles of Indian Animals with a view to search for 11 and 12 oxygenated steroids needed for synthesis of anti-arthritis compounds related to "cortisone".	Dr. A. M. Mehta, D. Phil
2	Indian Council of Medical Research, New Delhi.	Researches on the Purification and Chemical Structure of Immune Protein and Active Immunizing Agents.	Shrimati Shanta S. Rao M.Sc.
3	Unichem Laboratories, Bombay.	Researches on Casein Hydrolysate.	Dr. K. C. Thadani, B.Sc (Hons.) Ph.D.

Besides, there were two other Government Research Scholarships and they were held by the following candidates:—

1. Kumari K. M. Pavri, M.Sc.
2. Kumari K. M. Sethi, B.Sc.

In view of the excellent laboratory facilities in the Institute the Government of Bombay authorised the entertainment of Science students on payment of fees for taking their post-graduate degree courses by research, and the following candidates were admitted under this scheme:—

1. Kumari R. S. Vakil, B.Sc.
2. Shri S. V. Nabar, M.Sc.
3. Shri P. Y. Dighe, B.Sc.
4. Shri K. V. Tonpi, B.Sc.
5. Shri S. M. Vaidya, B.Sc.
6. Shri K. Vasant Pai, B.Sc.
7. Shri N. V. Bhagwan, B.Sc.

XV. Income and Expenditure.

Receipts.		Expenditure.	
	Rs.		Rs.
XXVII—Medical—		38—Medical—	
Sale Proceeds of Sera and Vaccines ...	20,58,066	H-Bacteriological Laboratories—	
Other Miscellaneous Receipts ...	19,177	H-1 Haffkine Institute,	
		H-2 Pasteur Institute.	
		Pay of Officers ...	2,15,638
		Pay of Establishment ...	3,87,121
		Allowances etc. ...	3,13,044
		Contingencies ...	10,44,471

PART II.

RESEARCH WORK.

I. Nutritional Requirements of *P. pestis*.

(WAGLE AND GOVIND)

(1) *Chromatographic Study of Casein Hydrolysate.*

It has previously been reported that treatment of the acid digest of casein which we use for preparation of vaccine with activated animal charcoal considerably enhances the nutritional value of the hydrolysate for the growth of *P. pestis*. While in the case of unadsorbed medium, the viable count of the plague organisms amounted on an average to 400 million per millilitre, in the case of adsorbed and decolorised medium the viable count rose on an average to 700 to 1000 million organisms per ml. It was considered that charcoal acted either by removing some inhibitory factor from the medium, or by setting right an imbalance in the amino-acid composition of the medium by differential adsorption, or by acting as reducing agent in the medium. The first line was investigated by obtaining a water extract or Methanol extract of the adsorbed material and testing its effect on the growth of the plague bacilli after adding the extract to the adsorbed casein hydrolysate medium in different quantities. No inhibitory action was found to be present against *P. pestis*.

In order to see if any amino-acid was responsible for the inhibitory action, the effect of the addition of different amino-acids to a synthetic medium which did not by itself contain any amino-acid was studied. No inhibitory or enriching effect was noticed in the case of any of the 21 amino-acids tested. Since these experiments did not give any positive result this problem was studied this year by paper chromatography.

Chromatographic study of the amino-acid constituents of the adsorbed and unadsorbed liquid casein hydrolysate medium was done. For this about 25-50 microlitres of the respective hydrolysates were placed at one corner 1 cm. from either edge of a sheet of Whatman filter paper No. 1 and allowed to dry in air. The paper was then folded into a cylinder and placed in a petridish containing a mixture of Butanol, acetic acid and buffer and allowed to develop 10-12 hours. The filter paper was dried, folded into a cylinder in a direction perpendicular to the former, and placed in a petridish containing phenol (purified) and buffer, and allowed to develop 8-10 hours. The paper was again dried and amino-acid spots indicated by spraying with a 0.2% solution of ninhydrin in butanol saturated with water, and drying at 80°C. The identification of the spots was done by comparison with the R_f values of pure synthetic amino-acids in the two developing solvents. The following amino-acids could be identified from the chromatogram: (1) lysine (2) aspartic acid (3) histidine (4) glycine (5) serine (6) arginine (7) threonine (8) alanine (9) glutamic acid (10) proline (11) tyrosine (12) methionine (13) valine (14) isoleucine (15) leucine (16) phenyl-alanine. It was observed that qualitatively there was no difference in the two hydrolysates as far as could be noted from the location of the amino acid spots. Quantitative assay of the amino-acids in untreated and charcoal treated hydrolysates are in progress.

(2) *Studies of Possible Reducing Action of the Charcoal.*

Since the chromatographic analysis of the liquid casein hydrolysate medium did not suggest removal by charcoal of any amino-acids inhibitory to the growth

of *P. pestis*, experiments were undertaken to see if charcoal possibly acted as a reducing agent in the medium. It is known that nutrient agar which is not so suitable a medium for the growth of *P. pestis* becomes quite suitable by addition of rabbit or human blood to it. If a small inoculum which is capable of producing 200-300 colonies on blood agar, is inoculated on nutrient agar no growth takes place. Experiments were, therefore, carried out to see if the incorporation of charcoal or addition of water extracts of charcoal to the nutrient agar would help the growth of *P. pestis* on this medium. Incidentally experiments were also done by adding to nutrient agar charcoal which was already used once for adsorption and decolorisation of casein hydrolysate medium, or water extract of such used charcoal. The effect of treatment of the broth (which is used for the preparation of nutrient agar) with charcoal was also studied.

The results obtained are given below in table.

Inoculum.	Standard agar.	Blood agar.	Standard agar plus 0.5-1% charcoal.	Standard agar where the broth was treated with charcoal.	Standard agar plus water extract of charcoal.	Standard agar plus 1% used charcoal.	Standard agar plus water extract of used charcoal.
1 ml. loop of 24 hour broth culture.	No growth	Confluent growth.	Confluent growth.	Confluent growth.	Confluent growth.	No growth	No growth
1 ml loop 10 ₁ dilution of culture.	Do. ..	Do. ..	Do. ..	Do. ..	Do. ..	Do. ..	Do.
1 ml loop 10 ₂ dilution.	Do. ..	Almost confluent growth.	Almost confluent growth.	Almost confluent growth.	Almost confluent growth.	Do. ..	Do.
1 ml loop 10 ₃ dilution.	Do. ..	About 150 colonies.	About 150 colonies.	About 150 colonies.	About 100 colonies.	Do. ..	Do.
1 ml loop 10 ₄ dilution.	Do. ..	30-40 colonies.	30-40 colonies.	30-40 colonies.	20-30 colonies.	Do. ..	Do.
1 ml loop 10 ₅ dilution.	Do. ..	6-7 colonies.	5-6 colonies.	4-5 colonies.	2-3 colonies.	Do. ..	Do.

Results show that incorporation of charcoal or water extract of charcoal helps the growth of *P. pestis*, almost to the same extent as addition of blood does, while charcoal which is used once for adsorption and decolorisation is found to lose this effect after one use.

These results may mean that charcoal either acts as a reducing agent or supplies some factor which is favourable for the growth of *P. pestis*. The possibility of reducing action has already been suggested by Rockenmacher et al (1952) who has emphasised the contrast between the beneficial effects of aerobic conditions on growth in liquid medium and the beneficial effects of reduced atmospheric pressure of solidified media in the absence of blood tissue extracts, reducing agents or hematin. Herbert (1950) has also previously explained the inability of small inocula of *P. pestis* to grow on solid media on the basis of inhibition due to hydrogen peroxide which is not decomposed by the "catalase" present in *P. pestis* if the inoculum is

(3) *Amino Acid Requirements of P. pestis.*

The chromatographic analysis of casein hydrolysate medium opened up the way to study the amino-acid requirements of *P. pestis*. Chromatographic analysis of the amino-acid composition of the adsorbed casein hydrolysate medium was already. In order to determine specifically which amino acid or acids are utilised by *P. pestis* during its growth in the casein hydrolysate medium, the casein hydrolysate medium in which *P. pestis* was grown for 14 and 30 days respectively was studied chromatographically. On comparison of the spots obtained with the fresh casein hydrolysate and those obtained with casein hydrolysates in which *P. pestis* was grown, it was found that proline spot was absent in the chromatogram of casein hydrolysate in which *P. pestis* was grown, thus indicating that Proline was completely utilised during the growth of *P. Pestis*. There was no observable difference in other amino acid spots except that the intensity of the leucine spot was very much reduced in the culture fluids. It is proposed to make a quantitative study of the amino acid requirements of *P. pestis* next year.

It is proposed therefore to continue the quantitative study of the amino-acid requirements for the growth of *P. pestis* as also to study the mechanism of the action of charcoal in enhancing the growth of *P. pestis*.

II. Field Trials with the New Diphtheria Prophylactic-N. A. F. T.

(LAHIRI).

During the year and the previous year, 80 adults and 433 young children were immunized with different diphtheria prophylactics under controlled conditions so as to compare the N.A.F.T. (natural adjuvant factor toxoid) with the A. P. T. In case of the adults, the quantities of antitoxin, which were produced in them by single doses of the prophylactics were determined to measure their response. In case of children, however, only the Schick Conversion Rates following single doses of the prophylactic were determined to compare the antigenicity of the prophylactics. The reactions suffered by each individual subject were also carefully noted.

The most noticeable property of the new prophylactic N.A.F.T. as revealed by these field trials, was that it was almost completely free from reactions. It did not produce any reaction at all in the children. Even in the adults the reactions were very few and very mild, and were, therefore, negligible. The A.P.T., on the other hand, produced bad reactions, not only in the adults but also in the children.

Though the N.A.F.T. did not produce any reactions, it was still highly antigenic. In these field trials, it showed itself to be as antigenic as the A.P.T. used as controls.

III. Anti-Human Rabbit Serum Floccule in the Treatment of Allergic States.

(LAHIRI).

Encouraged by the reduction in the incidence of horse serum reactions since anti-human horse serum had been incorporated with the therapeutic sera, it was resolved to try the effect of anti-human anti-body in the treatment of other allergic states of man. For these trials, anti-human serum was prepared in rabbits. In order to purify and concentrate the anti-body, the rabbit anti-body was flocculated with human serum, the floccule was suspended in an excess of human serum and then freeze-dried. Just before use the freeze-dried floccule was re-constituted and then stored in the cold till the suspension was used up completely.

Two groups of patients were taken up for these trials—one comprised chiefly those suffering from naso-pharyngeal allergy, while the other consisted of those whose allergy manifested itself in the form of some dermal involvements. The results obtained in the first group were quite convincing of the efficacy of treatment. The second group did not show such convincing results, but the patient had considerable relief from the suffering.

IV. Naturally-Developed Diphtheria Antitoxin Titre of Random Population

(LAHIRI AND MORE)

Two hundred and ninety four adults, aged between 20 and 50 years, were taken up for the quantity of naturally developed diphtheria antitoxin present in their sera. None of these had ever had any artificial immunization against diphtheria.

Out of this number 80 adults, aged between 25 and 50 years, were members of the staff of the Haffkine Institute. The geometric mean antitoxic titre of these was 0.07 units. The second group was drawn from amongst the blood donors who were bled at the Haffkine Institute blood bank. These were mostly poor people who donated their blood for the sake of money. They were aged between 20 and 40 years, and showed a geometric mean antitoxic titre of 0.24 units. The third group consisted of orphaned inmates of a missionary institution. Perhaps the youngest people, aged between 20 and 25 years, had the most squalid existence during their childhood. Proportionate with this squalor, and consequent heavy exposure to the risk of infection, they showed a geometric mean antitoxic titre of 0.29 units—the highest of the lot.

All of these adults must have developed diphtheria antitoxin through repeated exposures to infection. The Haffkine Institute volunteers belonging to middle class educated families were likely to be the least exposed, of all the three groups examined in this study, and, hence, proportionate with this least exposure, had the smallest quantity of natural diphtheria antitoxin in their sera. The people of the two other groups, living a more squalid existence than the Haffkine Institute volunteers, had nearly 4 times as much antitoxin as that developed by the Haffkine Institute group consisting of middle class people. Thus, the quantity of naturally developed antitoxin seemed to be proportional to the social condition of living and, hence, to the frequency of exposures to diphtheria infection.

The large quantities of natural antitoxin, present in such a random population, indicated the prevalence of diphtheric infection in this country. Perhaps it was the height of the natural immunity in the exposed, and the mildness of infection which has so far prevented disastrous epidemics to appear.

V. Electrophoretic Pattern of Polyvalent Anti-Snake-Venom Serum.

(LAHIRI AND RAO).

Like the antitoxins, the venom neutralising antibody is precipitated by 30 per cent. and 50 per cent. saturation with ammonium sulphate. The anti-venom serum is, therefore, purified and concentrated in the same way as the

It is a common experience that horse derived antitoxic sera show an additional peak, which is not present in the normal serum. This peak has been given different names, such as T-component, beta-2-globulin, or gamma-1-globulin. We too found the same peak in antitoxins of our make. But the polyvalent anti-snake venom serum did not show this peak. It showed only the three globulins present in normal serum, with slight excess of gamma-globulin.

VI. Effect of Lipid Extraction on the Antibodies of Concentrated Immune Horse Serum Globulins.

(MORE AND RAO)

It was observed that lipid extraction carried out at room temperature under the conditions given below did not involve any denaturation nor any loss of antibodies in the immune horse serum globulin fraction, there was actually an increase in the antibody titre per mg. of protein nitrogen.

The pressed cake of serum pseudoglobulin fraction precipitated by ammonium-sulphate between 28 per cent and 50 per cent saturation was dried in a desiccator to less than 1 per cent. moisture. The dry cake was powdered and extracted 3 times with a 1:1 mixture of dry freshly distilled ether and absolute ethanol. The precipitate was finally washed with ether, de-etherised by exposure to air and then dialysed to remove ammonium sulphate. It was found that after dialysis there was a large amount of precipitate which could be removed by dilution and adjustment of pH to 5.5.

Seven lots of anti-diphtheric horse plasma were treated in this manner and the antibody titre of the extracted sera determined by flocculation test. There was no loss but an increase of 10 to 40 per cent. in antibody titre per mg. protein N. The precipitate which appeared after dialysis thus consisted of non-antitoxic globulin. Electrophoretic analysis of extracted and unextracted samples showed that in the case of extracted sample the peak representing alpha or lipid rich globulin was absent. The increase in the antibody titre, was thus due to removal of alpha-globulin which had no antibodies associated with it.

VII. Micro Electrophoresis on Paper.

(RAO)

A simple and inexpensive apparatus was set up which consisted of a glass refrigerator tray divided into 5 compartments with glass partitions. The glass partitions were made water tight by pressing plasticine. The central compartment was 14 cm. wide and the other four compartments were each about 4 to 5 cm. wide. Carbon electrodes were placed in the two extreme compartments. These two compartments communicated with their adjacent compartments by the help of wicks placed over the partitions dividing them. All compartments except the middle one were filled with buffer solution. The solutions were levelled in these four compartments by putting syphon tubes. Five paper strips each 4 cm. wide and about 20 cms. long were wetted and placed over the central compartment with their ends dipping in the buffer solution across the partitions. The syphons were removed and a tiny drop of protein was placed on each of the filter papers. Direct current of 10 milliamp. was applied through the carbon electrodes. Electrophoresis was carried out overnight for 16 to 20 hours. The papers were then dipped in bromophenol blue solution when the protein absorbed the dye. Excess dye was washed with 0.5 per cent acetic acid.

Advantages of paper electrophoresis over free electrophoresis are, firstly, as small as 0.01 cc. of protein solution is sufficient for an analysis. Secondly, as many as five different estimations can be carried out simultaneously. The results by the two methods agree fairly closely.

A number of proteins have been analysed by this method including venoms, horse serum products and bovine lens proteins.

Among the different qualities of papers tested, whatman No. 31 proved to be the best. Next in order were whatman No. 3 and No. 1.

VIII. Electrophoretic Analysis of Proteins.

(RAO)

Electrophoretic analysis of the venoms of the four poisonous snakes of India viz. Cobra, Krait, Russell's, Viper and Saw-scaled Viper were analysed by electrophoresis in the Tiselius apparatus at pH 8.6 and ionic strength 0.1 in barbiturate buffer and at pH 7.2 and ionic strength 0.1 in phosphate buffer. Attempts are being made to correlate the different electrophoretic peaks with the several physiologic constituents of the venoms.

Bovine whole lens, aqueous, and vitreous proteins were analysed electrophoretically at pH 8.6 in barbiturate buffer. Each of the three proteins contained three components. The corresponding three components had identical electrophoretic mobilities indicating the possible identity of these in the three proteins.

Experiments were carried out to purify and isolate the components of bovine whole lens using the electrophoretic method as guide. It was found that the fastest moving component had an isoelectric point of pH 5 and could be precipitated in fairly pure form at this pH.

Horse anti-diphtheria pseudoglobulin was subjected to electrophoretic analysis before and after lipid extraction. It was found that albumin was totally absent in the lipid free pseudoglobulin.

IX. Studies in Concentration of Diphtheria Toxoid.

(SHANTA S. RAO)

Diphtheria toxoid pooled and dialysed, 37 Lf/cc. and containing 0.063 mg nitrogen per Lf. was purified by the different methods and also by the tryptic digestion method. Results are given in table below :

Serial No.	Method.	Yield percent.	Mg. N per Lf.
1	Ammonium sulphate method of Levene et al. ...	70	0.0069
2	Holt's method using adsorption with cadmium chloride ...	63	0.00152
3	Polyphosphate method of Jacob et al ...	70	0.0015

The enzyme digestion method was found to give toxoid of highest purity. Similar results were obtained with another lot of toxoid.

The antigenicity of these purified samples is now being tested.

X. Studies on Homogeneity of Antigens by the Semi-Solid Precipitin Technique.

(KULKARNI AND RAO)

Antigen antibody reaction in semi-solid media was studied using the recent technique developed by Oudin. The technique is simple and is very useful in getting an estimate of the number of antigens in a solution and in finding the purity and homogeneity of proteins.

We have used the technique in the study of antigens in the venoms of four poisonous snakes of India, viz : Cobra, Krait, Russell's Viper and Saw-scaled Viper, and also in other antigens, bovine lens protein fractions, typhoid vaccine, plague vaccine and human B-group ovarian cyst fluid.

From the results we can conclude the following :—

1. At least 8 antigens have been detected in each of the four Indian snake venoms.
2. There are common antigens in bovine lens, aqueous and vitreous. The alpha fraction isolated from bovine lens proteins is free from beta protein albumin and gamma-globulin fraction.
3. At least two antigens were detected in TAB vaccine.
4. Seven antigens could be detected in supernates of plague vaccine, two of which are in large amounts.

XI. Quantitative Studies in Antigen-Antibody Reaction in Agar Gel.

(KULKARNI AND RAO)

There appeared to be direct relation between the concentration of antigen used and the position of band of precipitate, while studying the antibody-antigen reaction by semi solid precipitin technique.

It was noticed on plotting the graphs that the position of the band was directly proportional to the logarithm of concentration of venom in milligrams per c.c. The relation obtained satisfied the equation :

$Y=mx+c$ for a straight line.

The values obtained from the statistical mean for the position of the theoretical line were found to lie along the straight line obtained graphically previously, and thus, supporting the relation obtained.

Equations for the lines showing such relation obtained using the four types of venom considering only one Major band in each are given below :

1. Cobra Venom Band No. 2 ... $Y=0.8X+0.65$
2. Russell's Viper Venom Band No. 1 $Y=0.66X+0.882$
3. Krait Venom Band No. 1 ... $Y=0.615X+1.158$
4. Echis Venom Band No. 1 ... $Y=1.14X-1.15$

XII. Comparison of Lethal Effects of the Venoms of the four Common Poisonous Snakes of Indian with a view to Studying their Physiological differences.

(KULKARNI AND RAO)

Experiments were carried out to determine the relationship between the intravenous and subcutaneous MLD's of venoms of Cobra, Krait, Russell's Viper, and Saw-scaled Viper.

The results obtained in mice weighing 20g as experimental animals are tabulated below :

Serial No.	Venom.	MLD in mg I/V route.	MLD in mg S/C route.	Ratio subcutaneous.	
				Intravenous.	
1	Cobra Venom	...	0.00934	0.0111	1.18 : 1
2	Krait Venom	...	0.00833	0.025	3 : 1
3	Echis Venom	...	0.0166	0.20	12 : 1
4	Russell's Viper Venom	...	0.00833	0.5	60 : 1

It was, thus, seen in a general way that the venoms of Colubrids was nearly equally lethal by the two different routes, while the venoms of Viperines killed mice in much smaller quantities when administered intravenously than when given subcutaneously. The preponderance of neurotoxins in Colubrids and haemotoxins in Viperines explains the difference. The haemotoxins would naturally act more when introduced directly into circulation.

Ld 50 and CLD of Cobra venom by Reed and Muench Method.

Cobra venom sample from a large pooled lot was used for this experiment. LD 50 and CLD of Cobra Venom was estimated using Reed and Muench method by subcutaneous route as well as by intravenous route.

White mice weighing 20 g were used for this experiment. The results were calculated graphically in each case.

Results.

Route of injection.				LD 50 value in mg.	CLD value in mg.	Ratio LD50 Sub	Ratio CLD Sub
						I/V	I/V
Intravenous	...	...	...	0.0082	0.011	1 : 1.2	1 : 1.09
Subcutaneous	...	...	...	0.00943	0.0125		

XIII. Pulicidal value of Pyrethrum.

(DEORAS)

25 experiments were done to test the pulicidal value of Pyrethrum in the powder formulation. This formulation was made either from the extract or from crushed flowers mixed with China clay. Piperonyl butoxide was tried as a booster to see if it enhanced the residual effect. The experiments were done in closed glass jars and also in W-shaped artificial rat burrows. Twenty fleas from a mixture of *X. chepis* and *X. astia* were allowed to contact the insecticide for 15 minutes and then removed to clean tubes for observation up to 24 hours. Some of the modern insecticides like DDT, BHC, Chlordane, Dieldrin and Parathion were also tried in powder formulation to compare the residual effect of the above powders. Some of the significant observations giving a residual effect, lasting upto more than two months in certain experiments are recorded at Table I.

Solution and emulsion formulations of Pyrethrum were also tried. They gave better immediate knock-down effects as compared to powder formulations, but their residual effect seems to be of much shorter duration. The experiments are being continued. However the results so far obtained show that the powder formulation of Pyrethrum (extract) with Piperonyl butoxide gives results comparable with DDT, BHC Parathion, Dieldrin, and Chlordane powder formulations for periods shown in the table.

TABLE No. 1. (20 fleas were used each time in the experiments. Only 15 minutes contact was allowed to the fleas and they were then kept for observation in a clean tube for 24 hours).

Name of the product used.	Concentration.	Method of application.	% Knock-down on the 1st day.		Residual Effect % Knock-down on		
			6 Hrs.	24 Hrs.	No. of days	6 Hrs.	24 Hrs.
I.							
POWDERS.							
Pyrethrum Extract 2% plus China Clay 1:10	25 mgm per sq. foot.	Dusted in jars kept closed.	100%	100%	111th day.	0%	100%
Pyrethrum Extract 2% plus 20% Piperonyl Butoxide plus China Clay 1:10.	Do.	Do.	100%	100%	119th day.	20%	100%
Do.	Do.	Do.	100%	100%	113th day.	30%	100%
DDT 10% in China Clay.	Do.	Do.	20%	100%	113th day.	5%	100%
Do.	Do.	Do.	35%	100%	88th day.	6%	100%

TABLE No. 1—*contd.*

Name of the product used.	Concentration.	Method of application	% knock down on the 1st day		Residual Effect Knock-down		% on	Remarks.
			6 Hrs.	24 Hrs.	No. of days	6 Hrs.	24 Hrs.	
II.								
Pyrethrum Extract 2% plus 20% Piperonyl Butoxide plus China Clay 1:10.	Do.	Do.	75%	100%	88th day.	8%	100%	
B. H. C. 10% China Clay.	Do.	Do.	85%	100%	88th day.	0%	90%	
Chlordane 5%	Do.	Do.	30%	100%	71st day.	0%	100%	
Dieldrin 10%	Do.	Do.	30%	100%	71st day.	0%	100%	
Parathion 14%	Do.	Do.	15%	100%	66th day.	10%	100%	
III.								
Pyrethrum powder 100 mesh plus China Clay 1: 10 plus 20% Piper only Butoxide.	Do.	Do.	45%	100%	66th day.	0%	100%	
Piperonyl Butoxide plus China Clay 1:10.	Do.	Do.	20%	100%	39th day.	0%	50%	
China Clay Control.	Do.	Do.	0%	0%	39th day.	0%	10%	

XIV. Rodenticidal value of 'Dethmor'.

(DEORAS)

Preliminary laboratory and field experiments were done with Dethmor baits supplied by Messrs. Standard Chemical Company of Bombay. Dethmor is a trade-name given to a brand of (Warfarin) anti-coagulant rat poison discovered by the Wisconsin Alumni Research Foundation of U. S. A. The baits were made by mixing Dethmor with "Wari" (*Panicum maliaceum*) in the proportion of 1:19.

Laboratory experiments were done with *R. rattus*, *R. norvegicus* and *Gunomys kok.* weighed rats were kept separately and fed on the poison baits in the first experiment

consumed only 1/6 before it died. Along with the normal food in the 2nd experiment, the amount of bait consumed was nearly the same as before but the deaths took place after 12, 8 and 6 days respectively.

The field experiments were done for 21 days over 28·984 acres of the Instituite area. 54 bait cages were used and the bait in them was weighed and replenished each day. The maximum amount of baits consumed in a single day was 213 gms in one cage, while the total amount of baits eaten during the entire period was 53 lbs. The rats began to die after the 5th day and the total number of rats dead during the period that showed typical internal haemorrhage were 33, of which 17 were *R. rattus*, 4 *Gunomys kok*, 8 *Bandicota malabarica*, 3 *Mus musculus* and 1 *Suncus sp.*

The baits cost Rs. 2-4-0 per lb. The non-recurring cost of cages, locks, and trays was Rs. 147-8-0. One cooly and one Supervisor were in attendance all along the period of experiments. Two cats and two crows were also picked up dead showing internal haemorrhage during these operations.

XV. Venom Tolerance in some Poisonous Snakes.

(DEORAS AND RAO)

Seven-day old Russell's viper snake killed a white mice weighing 20 gms. in 20 minutes, and a fifteen-day old killed the same in 4 minutes. As these young snakes were biting each other without any ill effect, experiments were done to see the amount of tolerance in these snakes and adults *Echis carinatus* to four kinds of adult snake venom. The venom solutions were prepared from lyophilised venom which was then injected subcutaneously into 3 to 4 week old Russell's viper young ones weighing about 8 to 12 gms. The mouse lethal doses were worked out on groups of mice weighing 20 gms. and the dose desired to be given was contained in 0·5cc. of the venom solution. The results of these experiments indicate that Russell's viper young ones could tolerate upto 5 mgm. of Cobra, Krait and Russell's viper venom and only 2 mgm. of *Echis* venom. *Echis carinatus* weighing about 12 to 14 gm. were seen to tolerate upto 25 mgm. of Cobra, Russell's Viper and Krait venom and upto 37·5 mgms. of *Echis* venom.

It is to be investigated whether this tolerance is due to some neutralising factors in these snake sera either of the anti-body or of specific inhibitors type. Work in this direction is in progress.

XVI. Chloromycetin in Typhoid Fever.

(DOGRA AND MODI)

Laboratory diagnosis of typhoid fever in India has always presented considerable difficulty owing to the disease being endemic and consequently cases atypical. Also, patients present themselves late in the course of disease and often after considerable medication. With the advent of antibiotics, difficulties have further increased and particularly so when Chloromycetin is administered. Under the "Typhoid Scheme" of the Government of Bombay, this Institute deals with a very large number of samples of blood for culture and triple widal test for typhoid. It becomes difficult to exactly state the significance of the results obtained. In order to clarify the position, it was considered necessary to investigate the effects of Chloromycetin in definite culture-positive typhoid cases, on blood culture and Widal as well as

study simultaneously control culture-positive cases treated on symptomatic lines, but without Chloromycetin. This was made possible with the courtesy and co-operation of the staff of the Hurkisondas Hospital and Arthur Road Hospital for Infectious Diseases in the City.

Complete clinical records were obtained. Repeated blood culture and triple Widal test was made. Urine and stool were cultured for presence of Typhoid group of organisms at regular intervals and continued during convalescence. So far 37 Chloromycetin treated and 12 control cases have been investigated.

These cases may be considered as moderate and moderately severe cases amongst poor class of patients. They were all culture positive. Chloromycetin was administered differently by three different physicians but in sufficient total quantity. Some of the salient laboratory finding are summarized below.

Blood Culture.—Became negative for typhoid group 1-11 days after administration of Chloromycetin-majority in 3-4 days. In the event of relapse, it was positive again.

Triple Widal.—In severe cases, Widal rose above the original titre ascertained on admission. In 23 out of 37 treated cases, it showed no significant rise.

The work is in progress. It is hoped to study more cases with a view to ascertain mortality, relapse and carrier rates in Chloromycetin treated cases as well as control cases. It is evident, however, that once Chloromycetin is administered in adequate doses, the chances of getting a blood culture positive or a triple Widal diagnostically significant become remote.

XVII. Pregnancy Test.

(DOGRA AND MODI).

Routine pregnancy tests are an important feature of the activities of the Department of Clinical Pathology. During the year under review, over 500 cases were tested. Often clinician desires to know the validity of the test and we had no data to go by. It was, therefore, decided to follow up all the cases for which a pregnancy test had been done in the Institute. With the co-operation of the doctors in Bombay, we have been able to already receive information about 25 per cent. of the cases tested. These are analysed below :—

	Total.	Positive.	Negative.
Rat Test	52	25	27
Rabbit Test	38	12	26

endocrine disorders, mental causes, tumours, etc., etc. Amongst the positive cases in one case the result was positive within 4 days after the date on which menstruation was due. This investigation is in progress.

XVIII. A Haemorrhagic Septicaemia in Snakes.

(NARASIMHAM).

In an attempt to detect the aetiological agent causing mortality amongst some of the snakes in the Institute, a detailed study of 5 bacterial cultures isolated from the dead snakes was carried out. All these cultures were found to be pure and to belong to the group *Proteus*. From the morphological, staining, growth, biochemical and serological characters and mouse pathogenicity, these cultures were found to conform to *Proteus hydrophilus* (SANARELLI) with possible and slight strain variation.

This particular organism has been reported to be the causative organism of a severe and highly fatal haemorrhagic septicaemia in all cold blooded animals like fishes, frogs, toads, salamanders, lizards, snakes, fresh water eels, etc. When experimentally inoculated with this organism, mice, guineapigs, rabbits, dogs, cats, chickens and pigeons are reported to die of a plague-like disease. This disease is synonymous with the following.—

- (1) In frogs-Red Leg Disease.
- (2) In hatchery reared Brook-trout-Ulcer Disease.
- (3) In pikes-Red Sore.
- (4) In Snakes-Haemorrhagic Septicaemia (Camin 1948, Journal of Parasitology, Vol. 34-4).

The characteristic symptoms of this disease in all animals are, (a) slow cessation of all movements and reflex actions by the diseased animals (b) development of haemorrhagic spots under the skin on the ventral position and thighs and purulent sores in some cases (c) the duration of the disease is three to ten days. Death is often preceded by convulsive movements and by stoppage of the heart and symptoms of asphyxiation (d) a rise in infection in summer or hot months and fall during winter or cold months.

These symptoms were observed in infected snakes.—Russel's viper

XIX. Isolation of *M. Tuberculosis* from Sputum and Body Fluids.

(MHATRE AND MODI)

Whereas direct smear examination for presence of tuberculous bacilli is not always possible due either to small number of tubercle bacilli or presence of saprophytes and contaminants, and, whereas animal inoculation is tedious, expensive and time-consuming, cultural methods are wellknown for the detection of tubercle organisms in sputum and other body fluids. The two satisfactory methods of sodium hydroxide digestion and triiodium phosphate digestion have been recommended for prior treatment of the material before inoculations on Lowenstein-Jensen medium. These two methods were compared. Both methods were simultaneously used on 200 samples of sputum and 35 samples of urine, pleural fluids, etc., etc.

Results of the sputum samples are given below.

Technique.	Smear+ Culture+	Smear+ Culture—	Smear— Culture+	Smear— Culture—	Contaminations Results inconclusive.
Sodium Hydroxide ..	55	8	42+8	59	28 $\frac{28}{59}$
Trisodium phosphate ..	27	40	8+4	107	14

It would appear that sodium hydroxide concentration method is superior to the trisodium phosphate method. The trisodium phosphate method seems to show a retarding effect and to a lesser extent inhibiting effect on the growth of *M. tuberculosis*. Trisodium phosphate method, however, had a definitely superior inhibiting influence over the acid-fast saprophytes and contaminants.

XX. Isolation of enteric group of Organisms from Contaminated blood Samples. Comparative Study of D. E. C. and B. S. A. Media.

(MISS BHARUCHA AND MODI.)

We were confronted with a large number of contaminated blood samples from cases of suspected typhoid fever. These contaminants-spreaders and pyocyanous-made isolation of salmonella group difficult and tedious.

A pilot experiment to assess the value of the various modifications of Wilson and Blair medium showed that the modifications suggested by McCahey and MacCartney (1950) with 4 per cent. agar as the base was superior to the others. For the actual investigation the blood clots were first transferred to bile broth from which they were repeatedly plated at 24 hours intervals on nutrient agar and in the case of contaminated samples on D.E.C. To save both material and labour, the B.S.A. was employed only in cases where the D.E.C. showed gross contamination and hence there was a likelihood of the contaminants overgrowing the enteric organisms. By using the B.S.A. in addition to the D.E.C. in 628 samples, 6 per cent. additional cases could be recognised. It may be stressed that the B.S.A. was more inhibitory to contaminants and is of special significance when enteric organisms grow in association with *B. pyocyanous*. However, the results with B.S.A. plates are invariably delayed to 48 hours. Hence it is recommended that B.S.A. medium should be used as a subsidiary medium to the D.E.C., as a higher percentage of positive results could be obtained by using both the media than when only one was used.

XXI. Laboratory Aspects of Cases of Diphtheria in Bombay City.

(MISS BHARUCHA AND DOGRA)

Diphtheria occurs in the City of Bombay as an endemic disease. From time to time, samples of throat swabs are submitted for laboratory reports. The co-relation of clinical and bacteriological findings was deemed necessary, as in a number of cases a clinical case showed nonvirulent strain of *C. diphtheria* and vice versa. It

With the above in view, so far 28 clinical cases of diphtheria have been studied at the Arthur Road Hospital. Bacteriological studies were made to ascertain morphology, type and virulence. Of the 28 samples, 5 were diphtheroids. The rest, i.e. 23 were *C. diphtheria* of various types : 20 belonged to the mitis type having 4-7 mitis characters, 3 intermediate-like and none of gravis type.

For testing virulence or toxicity of the organism, the intracutaneous rabbit test and the invitro plate test were performed. Rabbit test in all the 23 samples showed positive virulence test, thus showing that the strains isolated were virulent.

Our experience with invitro plate test is still in its preliminary stages. The 25 stock strains tested in comparison with rabbit test showed that this test is reliable provided horse serum antitoxin was kept constant and the technique strictly adhered to.

This work is in progress.

XXII. Serodiagnostic Tests for Syphilis a Comparative Study.

(SAMANT)

Receipt of a very large number of blood sera from suspected cases of syphilis at this Institute afforded an opportunity to study the comparative sensitivity and accuracy of the Wassermann, Kahn, Meinecke's and V.D.R.L. slide flocculation tests.

Sera of 225 cases with known clinical signs of syphilis were selected and tested along with 838 cases in which syphilis infection was suspected. On these 1,063 samples, all the four tests mentioned above were carried out simultaneously. Results are tabulated below.

Syphilitic stage.	Total.	Result.	V.D.R.L.	Khan.	W.R.	M.K.R.
Primary	38	Negative ..	21	23	23	22
		Doubtful ..	1	0	0	2
		Positive ..	16	15	15	14
Secondary	110	Negative ..	37	38	38	40
		Doubtful ..	3	2	6	4
		Positive ...	70	70	66	66
Tertiary	77	Negative ..	43	45	43	48
		Doubtful ..	2	1	3	1
		Positive ...	32	31	31	28
Doubtful or Unknown ..		Negative ..	591	697	598	622
		Doubtful ..	5	34	28	27
		Positive ..	242	207	212	189
Total Sera tested ..	1,063	Negative ..	692	689	703	732
		Doubtful ..	11	37	37	34
		Positive ..	360	337	323	297

From the above table, it can be seen that (i) V.D.R.L. gives a high percentage of positive reactions in all stages of syphilis (ii) Kahn and W.R. give approximately the same number of positive and doubtful results in all the stages of syphilis. But the number of positive results given by Kahn test in secondary syphilis are slightly

higher than that given by W.R., (iii) M.K.R. shows the same reactivity toward primary syphilitic sera as the other tests. However, it does not show this high sensitivity towards the sera from secondary and tertiary syphilis, (iv) in the case of sera from doubtful or suspected cases of syphilis, the maximum sensitivity is shown by V.D.R.L., next in order come Kahn Test, W.R. and the last M.K.R.

A further comparative study of the flocculation test only, was also conducted to compare the three flocculation tests in vogue, namely, the Kahn, V.D.R.L. and Meinicke's tests. Sera of definite clinically syphilitic cases and those in which syphilitic infection was suspected were tested. The results are tabulated below :-

GROUP I.—*Sera with definite manifestations of syphilis.*

Syphilitic stage.	Total.	Result.	V.D.R.L.	Kahn.	M.K.R.
Primary	177	Negative .. 52 Doubtful .. 7 Positive .. 118	65 4 108	65 4 108	69 4 114
Secondary	232	Negative .. 65 Doubtful .. 11 Positive .. 156	72 3 157	72 3 157	75 5 152
Late or Tertiary	121	Negative .. 55 Doubtful .. 4 Positive .. 62	61 3 57	61 3 57	62 1 58
Total	530	Negative .. 172 Doubtful .. 22 Positive .. 336	198 10 322	198 10 322	196 10 324

GROUP II.—*Sera unaccompanied by any data concerning the evidence of syphilis.*

Syphilitic stage.	Total.	Result.	V.D.R.L.	Kahn.	M.K.R.
Doubtful or Unknown ..	1,681	Negative .. 1,021 Doubtful .. 59 Positive .. 501	1,037 88 454	1,037 88 454	1,075 56 450

GROUP III.—*Sera from patients suffering from diseases other than syphilis, viz., typhoid, typhus, malaria, leprosy, etc.*

Sera.	Total.	Result.	V.D.R.L.	Kahn.	M.K.R.
Non-syphilitic	408	Negative .. 369 Doubtful .. 15	367 22	367 22	372 16

It would appear from above data that the V. D. R. L. test is the most sensitive gives the least number of doubtful results, is the easiest to perform and most economic.

This work will be pursued during next year to cover a very much larger number of samples.

XXIII. Preservation of Rabies Seed Virus.

(DOGRA)

Work in connection with dried seed virus was continued during the year under review. Lyophilised rabbit brain rabies seed virus was tested for its titre for a further period. The results are tabulated below :—

Titre of Rabbit Brain Seed Virus after Lyophilisation.

Serial No.	Date	Number	Initial Titre	Soon after drying	Dried Material			
					2	4	6	8
1	5th April 1951	... 385-6/51	-6.19 10	...	-5.74 10	-5.12 10	...	...
2	26th April 1951	... 462/51	-5.60 10	...	-4.74 10	-4.62 10	...	...
3	17th September 1951	10022-24/51	...	-5.40 10	...	-4.65 10	-4.60 10	-4.62 10

Serial No.	Date	Number	After months					
			10	12	14	16	18	20
1	5th April 1951	... 385-6/51	-4.50 10	-5 10	...	...	...	-5.38 10
2	26th April 1951	462/51	...	-4.24 10	...	Material exhausted		
3	17th September 1951	10022-24/51	...	...	...	-4.62 10	...	...

It would appear that rabbit brain rabies seed virus can be preserved in lyophilised form and that if suitably processed should retain its potency for a long time.

The original lowering of titre, it was conjectured, could be minimised by snap-freezing the brain emulsion at -50°C to -70°C and subsequently drying the material. This has been done. The results are awaited.

XXIV. Lyophilised Rabies Vaccine.

(DOGRA)

It was reported in 1951 that a lyophilised rabies vaccine was prepared and tested. This vaccine was a sheep-brain chloroform-killed, milled and lyophilised product. Further tests showed that although this vaccine compared favourably well with Semple's vaccine prepared from the same sheep-brain and tested simultaneously, it was equally unstable and showed lower levels of potency on storage. Further work, however, must be done before drawing final conclusions.

It is proposed to further study this and a formaline killed, milled lyophilised sheep-brain rabies vaccine.

The work is in progress.

XXV. Immunity in Man after Treatment with Semple's Vaccine.

(DOGRA)

Anti-rabic treatment centre at this Institute attends to as many as 4 to 5,000 patients annually who apply for Pasteur prophylactic treatment. This afforded material for studying the immunity produced in man after treatment with the five per cent. sheep-brain carbolised anti-rabic vaccine (Semple's vaccine).

A mouse protection test was employed using the normal technique for such tests (*see* Annual Report Haffkine Institute, 1951).

Preliminary results obtained with 20 human sera of treated cases are given below. The number examined is small. It is proposed to continue this work.

Table showing results of mouse protection test with human sera.

Before treatment.		Within 3 months of Treatment.		Within 6 months of Treatment.		Within 9 months of Treatment.	
Case No.	Animal Result.	Case No. & Class of Treatment.	Animal Result.	Case No. & Class of Treatment.	Animal Result.	Case No. & Class of Treatment.	Animal Result.
5	0/5	1 III	5/5	7 III	5/5	13 II	5/5
6	0/5	2 II	5/5	12 III	5/5		
9	0/5	3 II	5/5	17 II	4/5		
10	0/5	4 III	5/5	20 II	5/5		
14	0/5	8 II	5/5				
		11 III	5/5				
		15 III	5/5				
		16 III	5/5				
		19 II	5/5				

Nominators denote mice survivors and denominators number of mice used. The controls (5 mice with each group of sera tested) showed 100% mortality.

It would be observed that whereas none of the sera before treatment showed any protective anti-body against rabies infection the sera of the patients treated with Class II and III treatments even upto 9 months showed enough anti-bodies to neutralise the mouse brain challenge Rabies fixed virus.

XXVI. Infantile Diarrhoea.

(MISS PAVRI)

features of such stools described by the other workers in this field outside India. 150 stools of diarrhoic children were studied with a view to finding the predominating pathogenic organisms. In over 50% cases *Proteus mirabilis* was isolated in large numbers. The organisms disappeared on cure.

This is the first time that evidence has been shown that *P. mirabilis* is able to cause infantile diarrhoea.

XXVII. Studies on Influenza.

(SOMAN AND DIGHE)

The work carried out refers to studies on two type B strains of influenza virus isolated in Bombay last year. Many more egg passages had to be carried out to adapt the strains to growth in the allantoic cavity. At the eighth egg passage, the virus strains could be fairly established in the allantoic cavity. Both strains were sent to Dr. C. H. Andrews, W.H.O. Influenza Centre, Mill Hill, London, for confirmation. The one strain isolated first failed to take in eggs there but the second one could be grown easily and was identified and confirmed as Type B influenza virus. Further this strain was studied along with other Type B strains and it was found to be distantly related antigenically to standard Type strain Lee and much closer to another type B strain Crowley isolated in the year 1946. In view of the recommended nomenclature of the newly isolated strains in different countries, our own strain would be labelled as Type B/India-1/51 and Type B/India-2/51. The first strain was once again sent to Dr. Andrews in London and it failed to grow. This strain is still maintained by egg passages and probably requires some more training before it is lyophilised, and sent to London. Since no ferret antisera were available for influenza work, antisera had to be prepared in fowls with the two standard strains of Type A and B influenza viruses and with the two locally isolated Type B virus strains.

Seven throat washings were collected during the year from suspected cases of influenza, paired sera being available only in four cases. All throat washings were declared negative after two egg passages and this was confirmed by the absence of any rise in the anti-body titre in those four cases. The technique followed in the isolation of influenza virus in eggs, or in the study of strains or serology in patients was as per recommendations of the report by WHO. Expert Committee on Influenza at its First Session in Geneva in September 1952.

Twenty paired sera from patients proved negative for the diagnosis of Q Fever by complement fixation test, were tested by haemagglutination-inhibition technique for influenza and only one of them showed a significant rise (8-fold difference) in anti-body titre against PR8 strain. The work of preparing soluble antigens and standardizing them for complement fixation tests is in progress.

XXVIII. Laboratory Diagnosis of Small-pox.

(SETHI AND SOMAN)

In tropical countries where small-pox occurs with seasonal periodicity, the clinical diagnosis should be quite obvious; sometimes, however, it becomes extremely difficult to differentiate a mild or modified case of small-pox (variola) from a case of chicken-pox (varicella). This is especially true of small-pox cases occurring out of season and on ships carrying passengers and crew. The Medical

Officer of Health of the City or that of the ship is obliged to seek aid of a laboratory to come to a diagnosis, prior to his taking public health measures. The results of laboratory tests may be available from within a few hours to three days of the receipt of the material. Three types of tests are available:—

- (1) microscopic examination of stained smears;
- (2) culture of the suspected material on the chorio-allantoic membrane of 10-12 day old fertile hen's eggs;
- (3) complement fixation tests using the suspected material as antigen along with a known positive anti-vaccinia-rabbit-serum.

Since October 1952, the first two methods were given a consistent trial with an idea to differentiate a mild or modified small-pox case from chicken-pox. Before collecting material from small-pox or chicken-pox cases, controlled experiments were carried out with known vaccinia infected material in rabbits and in eggs. Smears prepared from the scrapings of the base of lesions from small-pox and chicken-pox cases were stained, by Paschen's, Gutstein's and Giemsa's method for the presence of elementary bodies. In nine cases of small-pox, the provisional diagnosis could be made with ease, which was later confirmed by egg-culture. The value of microscopic examination cannot be overemphasized as the results are greatly controlled by experience of the worker in the technique of staining the smears. The microscopic differentiation of the elementary bodies of variola and varicella is mainly quantitative and hence the diagnosis could be only provisional and not absolute.

The most sensitive and specific test is the egg culture, although it takes 48-72 hours to give an answer. Varicella material inoculated into eggs does not produce any kind of lesions on the chorio-allantoic membrane. On the other hand, variola and vaccinia material on such a membrane produces obvious discreet whitish opaque lesions depending on the concentration of the virus inoculated. Egg culture was carried out in nine cases of small-pox and three cases of chicken-pox, using 11 days old eggs at the end of three days incubation at 37°C, chorio-allantoic membrane from each was harvested and the lesions studied.

This work is still in progress. The technique of complement fixation test for the diagnosis and differentiation of variola from varicella is under study.

XXIX. Studies on Avianized Rabies Vaccine.

(SOMAN AND KHEMANI.)

This vaccine contains active virus (Flury strain), modified and attenuated by 136 passages in one day old chicks by Johnson and further adapted to the chick-embryo by Koprowski. The virus is devoid of pathogenicity for dogs and dogs immunized with a single dose of this vaccine proved resistant to the inoculation of street virus. This vaccine was first made available to us through the kind courtesy of Dr. H. N. Johnson of the Rockefeller Foundation, U. S. A., for experimental purpose in May 1952 and it went successfully through four egg passages before the International Rabies Seminar took place in Coonor in July 1952. Each passage material in eggs was titrated in mice for its infectivity titre. The LD 50 titre in mice from each egg passage is shown in the following Table :

Egg Passage.

LD 50 Titre in mice.

The importance of preparation of such a vaccine was rightly stressed at the Rabies Seminar, and W. H. O. Rabies Consultant, Dr. Komarov, was deputed to work for a fortnight in the Institute and demonstrate the technique to other W.H.O. delegates. During Dr. Komarov's visit, the virus propagated in eggs was tested for its specificity using highly potent rabies antiserum from Dr. Lepine of Paris, with very good results. Further passages are being continued and it is proposed to look for a local strain of street virus which could be similarly adapted to embryonated eggs, for the preparation of avianized vaccine.

Technique for egg passage was as follows.

Chick embryos 7-8 days old are inoculated into yolk sac with a suspension of Flurry strain. 7-10 days later, the embryo is harvested, ground in saline and suspensions centrifuged. The suspension is tested in mice each time, the egg passage is done.

XXX. Studies on Q Fever.

(SOMAN AND KHEMANI).

The serological survey carried out on behalf of the World Health Organisation for the detection of Q Fever, was continued throughout the year. In addition to human and sheep sera tested, arrangements were made to obtain cattle sera from Aarey Colony through its Veterinary Surgeon. The technique employed in C. F. tests was as recommended by Dr. M. M. Kaplan in the information series W.H.O./200/17 Rev. of 25th June 1951. No result was recorded as positive unless the control serum gave reproducible results on each day of titration. Some of these human and sheep sera showing positive evidence of Q Fever anti-bodies have already received confirmation from Dr. George Slavin of the Weybridge Veterinary Laboratory, Surrey, England. Eleven human sera showing positive results were derived from five patients whose clinical histories were not particularly suggestive of Q Fever infection. Except one patient Reg. No. 68 from Table II the rest belonged to suburbs of Bombay.

The results of serological survey which commenced from October 1951 to this date are summarized in the following Tables I, II and in Table III.

(2) 34 Samples of pooled milk obtained from dairy cows and buffalos were inoculated intraperitoneally, 5cc. from each pool, into two guineapigs. All guineapigs were bled after 30 days and their sera were tested for complement fixing anti-bodies for Q Fever with negative results.

TABLE I.—*Results of complement fixation tests for Q fever on human, sheep and cattle sera.*

Sera.		No. tested.	No. positive.	Percent positive,	No. of sera showing positive results at different titres.					
					16	32	64	128	256	512
Human	...	367	11*	2.9	5	2	1	0	3	0
Sheep	...	376	39	10.3	18	15	5	1	0	0
Cattle	...	142	0	0	0	0	0	0	0	0
Total	...	885	50	13.2	23	17	6	1	3	0

*Eleven sera were derived from 5 human cases.

TABLE II.—*Complement fixation results on 11 human sera from 5 patients.*

Serial No.	Reg. No.	Locality.	Day of Fever.	C. F. titre.	C. F. titre as reported by Dr. Slavin.
1	5768	Borivli ...	24	64	16
2	5929	Bhandup ...	11	16	8
3	6618	Ghatkopar ...	15 22	32 16	Not sent.
4	68	Crawford Market...	23 30 36	256 256 256	Not sent. 256 16
5	2582	Kurla ...	11 18 26 27	16 32 16 16	Not sent. Do. Do. Do.

TABLE III.—*Results of C. F. Tests for Q Fever carried out in 1949.*

Sera.	No. tested.	No. positive.
Human sera of Pyrexial cases received for Widal test ...	739	3*
Human sera (Nonpyrexial) received for W. R. ...	99	0
Bandicoot sera from Poona ...	43	0
Total ...	881	3*

* Three sera showed non-specific reactions as reported by Dr. Lackman of Rocky Mountain Laboratory, Hamilton, Montana, U. S. A.

XXXI. Mouse Inoculation test for Diagnosis of Rabies.

(SOMAN AND KHEMANI).

It is a well-known fact that negri bodies cannot always be detected in men and

Since the demonstration that the intracerebral injection of rabies virus into white mice produces a typical and constant infection, the white mouse has become increasingly popular as a test animal. A positive microscopical diagnosis by finding out negri bodies is sufficient proof for the diagnosis of rabies but when such an examination proves negative, a pool of hippocampus, cerebellum and cerebral cortex is made and inoculated into four white mice, each intracerebrally with 0.03ml. of the inoculum. Therefore a negative finding does not exclude presence of rabies. Mice are observed for a period of 21 days before declaring the test as negative and all those mice are then destroyed. Mice usually come down with typical symptoms of paralysis etc. in 6-10 days. Each mouse brain is separately removed and impression smears made and stained to demonstrate negri bodies.

Diagnosis of rabies by microscopic examination of impression smears of brain and by the biological test in mice was carried out in parallel on 28 specimens. Positive evidence of rabies could be obtained only in 13 specimens by either of these tests and the rest were negative. The biological test was positive in all 13 specimens but the microscopic examination of the impression smears revealed the presence of negri bodies, i.e. positive evidence of rabies in 9 specimens. Thus the biological test has detected rabies virus in 4 specimens which would have been missed if only the impression smear technique was employed for the diagnosis of rabies in animals. It is now proposed to carry out this test as a routine procedure, especially on specimens reported to be negative by the impression smear technique.

XXXII. Liberation of Histamine by Snake Venoms from Striated Muscle.

(DUTTA AND ANANTANARAYANAN)

Quantitative study on the liberation of histamine from striated muscle (diaphragm) of the rat was carried out with the venoms of Cobra, Krait and Echis.

Details of the experimental techniques and the method adopted have already been reported (Nature, 169, 1064, 1952). The liberated histamine was biologically assayed on isolated guineapig ileum, which was sensitized to the respective venom. The results are given in the table below: Figures represent average of five experiments.

Release of histamine from rat diaphragm.

Venom.	Concentration of venom.	Time in minutes for which venom was allowed to act.	Percentage of histamine liberated.	Concentration of venom.	Time in minutes for which venom was allowed to act.	Percentage of histamine liberated.
Cobra (Naja Naja)	100 mg per ml.	5	33	50 mg per ml.	5	26
Do.	Do.	45	88	Do.	45	77
Krait (Bungarus caeruleus).	Do.	5	18.3	Do.	5	12.5
Do.	Do.	45	78.8	Do.	45	70.3
Saw scaled viper (Echis carinatus).	Do.	5	16.2	Do.	5	15.1
Do.	Do.	45	66.3	Do.	45	54.6

Of the three venoms studied, that of cobra proved to be the most potent liberator of histamine.

XXXIII. Stability of Vitamin B₁ in Oral Preparation.

(DUTTA, MEHTA AND ANANTANARAYANAN)

It has already been reported by us (Ind. J. Pharm., 1952, 14, 53) that both thiamine hydrochloride and mononitrate lose their potency in oral mixtures containing alcohol and certain minerals. The present work deals with the effects of Vitamin A acetate and B₆, ascorbic acid, Folic acid, Niacin, Calcium pantothenate, liver extracts, sodium salicylate and mixture of sodium salicylate and riboflavin on aneurine hydrochloride. Different solutions were prepared containing the above ingredients, the pH was adjusted to 4 and stored in amber coloured bottles at 25-30°C. These solutions were tested for vitamin B₁ from time to time both colorimetrically and fluorimetrically. At the end of six months it was observed that vitamin B₁ has deteriorated in mixtures containing vitamin A acetate, vitamin C or yeast extract. The results are given below :—

	Sodium Salicylate+B ₂	Sodium salicylate.	Niacin	Folic acid.	Vit. C.	Vit. B ₆ .	Yeast.	Cal. pantothenate.	Vitamin A.	Liver Extract.
Percentage of deterioration of B ₁ at the end of six months.	23	19.8	14.3	18.6	37.7	20.5	66.2	4	36.9	21.5

The adverse effect of ascorbic acid and vitamin B₁ was further studied by taking different concentrations of Vitamin C with a fixed amount of aneurine hydrochloride. The deterioration of vitamin B₁ was found to be directly proportional to the concentrations of ascorbic acid.

XXXIV. Comparative Nutritive Value of Cane-Sugar, Cane-Gur and Palm-Gur.

(GOVIND, KAMATH AND DUTTA)

The nutritive values of Cane-sugar, Cane-gur and Palm-gur were compared with that of starch when supplemented at a 36 per cent. level to different groups of rats fed a complete diet containing 15 per cent. casein, 4 per cent. salt mixture (Osborne and Mendel No. 185), 8 per cent. pea nut oil, 56 per cent. starch, 1 per cent. Shark liver oil (containing 1000 I.U. vitamin A and 100 I.U. of vitamin D per gram). In addition each animal received supplements of the vitamins of the B complex. The experiment was continued for three months. Daily food consumption and weight of animals twice a week were noted.

It was observed that compared with other carbohydrates tested, cane-sugar exerted the maximum growth-promoting effect. Cane-gur, palm-gur and starch came next in that order. Liver weight per 100 g. body weight was lowest and the Haemoglobin per 100 cc. blood highest in animals fed with palm gur. Though

XXXV. Analysis of Neera for its Mineral and Vitamin Contents.

(GOVIND AND KAMATH)

Twenty samples were analysed for mineral and vitamin contents. The average values per 100 ml. are given below :—

Iron	...	...	0.3683 mgms.
Phosphorus	...	...	9.167 mgms.
Calcium	...	...	Traces.
Vitamin C	...	...	7.015 mgms.
Vitamin B ₁	...	...	13.34 micrograms.
Vitamin B ₂ (Riboflavin)	...	...	Traces.

Other vitamins are present in insignificant quantities in fresh neera.

As neera begins to ferment and yeast grows, the yeast cells synthesize the B group of vitamins. Therefore, a practically fermented neera has a higher B vitamin content than fresh neera.

XXXVI. Lyophilisation of Neera.

(GOVIND, KAMATH AND DUTTA)

Lyophilisation of neera was done by the same process as is applied in this Institute for the lyophilisation of human plasma. Of the various substances tested for the prevention of frothing during lyophilisation, tannic acid was found to be the most suitable and the least objectionable. Addition of cafrlyic alcohol gave a characteristic odour to the product, whereas addition of 0.2 to 0.5 per cent. tannic acid completely prevented frothing and did not make any change in the flavour of neera. Analysis of the constituents of neera before and after lyophilisation showed that practically there was no deterioration in vitamin contents during the process. The average values are given below :—

Average Values,

		Iron mgms/ 100 ml.	Phosphorus mgms. 100 ml.	Calcium mgms. 100 ml.	Vitamin C mgms. 100 ml.
Before lyophilisation	...	0.367	9.222	Traces	7.660
After lyophilisation	...	0.350	8.980	Traces	6.710

XXXVII. In Vivo Absorption and Deacylation of N⁴ Acyl Derivatives of Sulphadiazine.

(SHIRSAT AND PATKI)

Numerous N⁴-acyl derivatives of various potent sulphonamide drugs (like slphathiazole, sulphadiazine, sulphamerazine and sulphamethazine) were synthesised by us (H. I. Report 1951) with a view to develop suitable derivatives of

sulphanilamido compounds, which would undergo slow but progressive hydrolysis to the parent compound *in Vivo*, thereby building up high and effective concentration of the drug, over an extended period of time, at the site of infection in the gastrointestinal tract, without giving rise to unduly high concentration of the drug in the circulating blood. A few selected number of the N⁴-acyl derivatives of sulphadiazine were chosen for *in vivo* study (with regard to their absorption, excretion and deacylation characteristics in white mice) in virtue of the well known antibacterial activity and low toxicity of sulphadiazine.

A group of 12 white mice (inbred Haffkine Strain) of average weight 20 gms. was used for each compound. The mice were fasted for 12 hours prior to feeding the drug.

0.5cc. of a stable suspension of the desired acyl sulphadiazine in gum emulsion (pH 7-8) equivalent to 10mg. of free sulphadiazine, was fed to each animal (0.5 gm/Kg body weight) orally in a single dose and a set of two mice was sacrificed after each interval of 1 Hr., 2 Hrs., 4 Hrs., 6 Hrs., 8 Hrs., and 24 Hrs., respectively after dosage, for the estimation of free and acyl sulphadiazine in Blood, Liver, Intestine and Faeces. During the experimental period following the dosage, no food was allowed but plain drinking water was provided freely.

For the estimation of free and acyl sulphadiazine in tissues, the original method of Bratton and Marshall using N (1-naphthyl) ethylenediamine dihydrochloride needed some modifications in order to obtain reliable results with difficulty hydrolysable N⁴-acyl derivatives of sulphadiazine. Leitz Photoelectric Colorimeter with filter D was employed for all the colorimetric comparisons.

The *in vivo* absorption and deacylation of 18 N⁴-acyl derivatives of sulphadiazine, including 4 aromatic acyl derivatives have been studied. (acetyl, propionyl, butyryl, valeryl, caproyl, heptyl, capryloyl, pelorgonyl, deconyl, lauroyl, palmityl, monochloacetyl, dichloracetyl, trichloracetyl, benzoyl, cinnamyl, phenyl, acetyl and ptohuoyl).

From the results thus obtained certain interesting features regarding co-relation between chemical structure and pharmacological properties have been brought out and it has been particularly shown that:—

(1) The acyl derivatives of sulphadiazine undergo deacylation *in vivo* both in the gastro intestinal tract and in the liver.

(2) The N⁴-acetyl compound is de-acylated to a very small extent but the next two homologues, viz. the propionyl and butyryl derivatives are deacylated to a great extent. The extent of deacylation however decreases thereafter with the increase in length of the acyl chain till finally the N⁴-palmityl derivative has been found to be deacylated almost to a negligible extent.

(3) While part of the acyl derivative is deacylated in the gastrointestinal tract, a certain portion thereof is absorbed as such through the gastrointestinal linings. The N⁴-acetyl compound is absorbed to a maximum extent and the degree of absorption falls down rapidly with the increase in length of the acyl chain. The so absorbed acyl compound subsequently suffers progressive deacyla-

(5) Among the chloroacetyl derivatives, the trichloroacetyl derivative is deacylated to the highest extent, next in order being the dichloroacetyl derivative, the monochloroacetyl derivative, being the least deacylated.

(6) The aromatic acyl derivatives are deacylated to a very small extent.

(7) On the basis of data obtained it has been tentatively suggested that for the treatment of bacterial infections of the gastrointestinal tract, N⁴-caprylyl derivative of sulphadiazine should be more safe and advantageous. In like manner the use of N⁴-caproyl and N⁴-heptoyl derivatives of sulphadiazine for the treatment of blood-borne bacterial infections and for the maintenance of therapeutic concentrations of free sulphadiazine in blood over an extended period of time has been suggested.

(8) The N⁴-propionyl sulphadiazine, under the dosage employed was found to be highly toxic to white mice.

Further work, with regard to similar studies with other derivatives of sulphadiazine are under way.

XXXVIII. Synthesis of 2-Hydroxy and 3-Hydroxy Sulphonamides and Benzoxazolyl Sulphonamides.

(SHIRSAT AND SARDESAI)

Following the report by Mayer (Bull. Acad. Med. 117, 727-35, 1937) that the activity of sulphanilamide is due to the formation of p-hydroxyamino benzene sulphonamide *in vivo*, a good deal of work has been done on the possible biological oxidation products of sulphanilamide with a view to see whether any of these products are responsible for toxicity or activity of sulphanilamide. Recently, Mayer's contention that p-hydroxyamino benzene sulphonamide is the biological oxidation product of sulphanilamide has been refuted by a number of workers and the possible biological oxidation products of sulphanilamide have been predicated to be 2-hydroxy sulphanilamide, 3-hydroxy sulphanilamide, p-amino phenol, 2-oxybenzoxazole etc. Out of these 3-hydroxy sulphanilamide is claimed to have been isolated from the urine of rabbits and patients fed with sulphanilamide (Williams R. T. Biochem. J. 40, 219, 1946). The compound when tested against streptococci *in vivo* was found to be inactive. Its *in vitro* activity was, however, different from that of sulphanilamide, since, whereas the activity of 3-hydroxy sulphanilamide was generally inferior to that of sulphanilamide, an increase in the inoculum substantially affected the antibacterial activity of sulphanilamide, which factor did not seem to affect the activity of 3-hydroxy sulphanilamide (Williams R. T. Biochem. J., 41, 1, 1947).

Now that sulphanilamide has been superseded by more active drugs like sulphathiazole and sulphadiazine, it was of interest to study the antibacterial and other biological activities of derivatives of sulphathiazole, sulphadiazine and other potent sulphonamides having hydroxyl groups introduced in the benzene ring. The synthesis of a number of 2-hydroxy and 3-hydroxy derivatives of potent sulphonamides was therefore undertaken with a view to compare their biological activities with the parent sulphonamides. The compounds will also serve as possible therapeutic agents.

Extending the application of this idea further, a number of 2-methyl benzoxazolyl-6-sulphonamides have been synthesised as potential chemotherapeutic agents.

In all about 50 compounds have been synthesised out of which 35 are reported for the first time. The antibacterial activity of these compounds is being investigated.

XXXIX. Chemotherapy of Intestinal Infections : Synthesis of Schiff's Bases of Sulphonamides.

(SHIRSAT AND SARDESAI)

The work of synthesising a number of Schiff's bases of sulphathiazole, sulphazine, sulphamerazine and sulphamethazine has been completed (H. I. Report 19). In addition similar derivatives of sulphaguanidine have also been synthesised. The following aldehydes and ketones were used for the synthesis :—

Aliphatic aldehydes.—Formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, iso-butyraldehyde, valerylaldehyde, iso-valerylaldehyde, hexaldehyde, heptaldehyde, crotonaldehyde and glyoxal.

Aromatic aldehydes.—Benzaldehyde, p-nitro benzaldehyde, o-nitro-benzaldehyde, 2:4 dinitro benzaldehyde, p-hydroxy benzaldehyde, m-hydroxy benzaldehyde, p-methoxy benzaldehyde, p-dimethylamino-benzaldehyde, cinnamaldehyde, vanillin and o-veratraldehyde.

Ketones.—Acetyl acetone, Acetonyl acetone, acetophenone, p-amino-acetophenone, benzoin, benzil, etc.

The compounds were synthesised by reacting an appropriate amine with aldehyde or a ketone either in presence or absence of a solvent. A few reactions were carried out in dilute hydrochloric acid.

In all about 145 compounds have been synthesised out of which 95 are being reported for the first time. All the sulphonamides and some of the aldehydes required for the reactions were synthesised in the laboratory.

Since the constitution of some of these derivatives reported in literature is a matter of controversy, particular attention was given to the study of this problem. formaldehyde derivatives were studied in greater detail and several interesting observations have been made in this connection.

Similar derivatives of p-amino salicylic acid have also been synthesised with a view to develop derivatives of p-amino salicylic acid having preferential action on gastrointestinal tract.

XL. Chemotherapy of Bacterial Infections : Synthesis of N¹-Heterocyclic Derivatives of Sulphanilamides.

(SHIRSAT AND BAPAT)

Following the discovery of sulphanilamide as a chemotherapeutic agent in treatment of bacterial infections, a number of sulphanilamide derivatives have been developed with a view to obtain drugs more effective and less toxic than the parent compound.

nilamide, particularly the less toxic bezimidazole nucleus, does not, however, appear to have been fully exploited and with a view to develop potent and less toxic heterocyclic derivatives of sulfanilamides, a number of N¹ (6-benzimidazolyl) sulfanilamides, having a variety of alkyl and aryl radicals at 2-position of benzimidazols ring have been synthesised. The work has also been utilised for studying the chemistry of benzimidazoles which is very fascinating indeed.

The alkyl groups introduced in 2-position of benzimidazole ring were methyl, ethyl, propyl, isopropyl, butyl, iso-butyl and amyl. The 2-alkyl benzimidazoles were prepared by heating a mixture of o-phenylenediamine and an appropriate aliphatic acid under reflux for suitable length of time. Under these experimental conditions it was observed that the yields of benzimidazoles were decreased with increase in mol. wt. of the acids. However, if the water formed during the reaction was progressively removed by distillation, the required benzimidazoles were obtained in almost quantitative yields irrespective of the acid used.

The aromatic groups introduced in 2-position of benzimidazole ring were phenyl, p-chloro-phenyl, m-chloro-phenyl, o-chlorophenyl, p-tolyl, m-tolyl, o-tolyl, β -pyridyl and r-pyridyl. The 2-aryl benzimidazoles were prepared by heating a mixture of o-phenylenediamine, the appropriate aromatic acid and 25 per cent. hydrochloric acid in a sealed tube at a temperature of 220-240°C for about 3 to 4 hours.

The 2-substituted (alkyl or aryl) benzimidazoles were nitrated and reduced with a suitable reducing agent and then condensed with p-acetylaminobenzene-sulfonylchloride or m-nitro-benzenesulfonylchloride in pyridine medium. The condensed products thus obtained were then hydrolysed or reduced to sulfanilamido or metanilamido derivatives respectively.

Several compounds thus synthesised will be subjected to in vitro and in vivo anti-bacterial activity against a variety of pathogenic organisms. It is also contemplated to investigate the extent to which the antibacterial activity of some of the active compounds would be nullified by PABA and related essential metabolites.

A few of the synthesised compounds are being studied for their probable anti-malarial activity with a view to develop newer series of antimalarial compounds.

XLI. An Improved and Rapid Method for the Determination of Serum Sodium.

(SHRIMATI VENKATARAMAN)

Chemical methods for the determination of serum sodium depend on its precipitation as the triple acetate with uranium and any divalent metal like zinc or magnesium. This is followed by the analysis of the carefully washed precipitate by gravimetric, titrimetric or colorimetric procedures. The manipulations involved in most of the published methods are slow and tedious, requiring precise chemical techniques unfamiliar to most technicians and thus increasing the possibility of errors. Often, by the time the result can be reported it would have lost much of its value to the clinicians who may require the information within a few hours after the specimen is submitted.

An extremely simple and reproducible method has been satisfactorily developed which consists of precipitating the sodium as the triple acetate with uranium and magnesium at a pH of about 5.2. The serum proteins are simultaneously precipitated by incorporating 75-80% v/v alcohol in the reagent.

The reagent has the following composition :—

Uranyl nitrate $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	...	...	10 gms.
Magnesium acetate ($\text{Mg Ac}_2 \cdot 4\text{H}_2\text{O}$)	..	..	8.6 gms.
Glacial acetic acid	..	..	30 cc.

The volume is brought to 200 cc. and enough alcohol, either absolute or 95% is added to make to 1 litre. The reagent is then saturated with reference to the triple salt by adding a few drops of normal saline. After 24 hours standing, the clear supernatant solution is ready for use and can be kept for many months without any change in the concentration of uranium.

0.1 cc. of the serum sample is added to 5 cc. of the reagent, accurately measured, and after precipitation of the proteins and the sodium as triple salt, the concentration of uranium in the supernatant is determined by Colorimetric procedure using potassium ferrocyanide. This is inversely proportional to the sodium precipitated. A standard curve is prepared by using various concentrations sodium ranging from 0.500 mg.%. In the absence of a photoelectric colorimeter, the readings can be made on the visual colorimeter.

This procedure is not only applicable to whole serum, but also protein free filterates or any other solution where the sodium content is required.

Some of the observations made during the course of this investigation would be of interest and use to other workers.

1. The reagent contains the minimum amount of the uranium salt thus avoiding much wastage of this costly and unavailable material.

2. Uranyl acetate used by all the previous workers has certain disadvantages viz. (a) its solubility is very low; (b) some of the samples available in the market do not have the expected degree of purity.

3. Uranyl nitrate can be very well substituted for the acetate even with advantage.

4. The use of zinc acetate has definite disadvantages if the uranium has to be estimated by colorimetry. The turbidity met with is due to the insoluble zinc ferrocyanide.

5. The use of magnesium acetate instead of zinc acetate is preferable.

6. The proportion of uranium to magnesium can be kept to a minimum of .01 mole to .02 mole per litre of the reagent.

7. The precipitation of the triple salt is complete in a few minutes; the subsequent centrifugation and colour development can be carried out after an hour or so.

By this procedure the manipulations are reduced to a minimum and a number of samples can be analysed in a couple of hours.

Complete details of this method will be published shortly.

XLII. Studies on Blood Glycogen.

(Submitted by V. V. RAMAKRISHNAN)

The source of blood glycogen is attributed to that of liver and it is claimed that the liver not only pours out glycogen into the blood but also accepts it as such for example when the assimilation of glucose or fructose by the liver takes place at an unusual speed or when the dissolution of liver glycogen occurs either by pilocarpin or by liver injury. Pilocarpin hyperglycemia is accompanied by decreased liver glycogen and increased blood glycogen. In adrenal hyperglycemia also the blood glycogen is found to be greatly increased.

No data is available of blood glycogen in various pathological conditions especially of the tissues which play a role in the carbohydrate metabolism—for example in liver diseases and the various hyperglycemic and glycosuric conditions.

A couple of years ago a micro method for blood glycogen was reported by Yamagata and we have found it to be quite workable with slight modifications. With this technique on hand it is planned—

- (1) to study the blood sugar and glycogen levels in liver diseases using clinical materials sent by hospitals.

- (2) to study the blood glycogen curve together with blood sugar curve in patients reporting for Glucose Tolerance Test.

- (3) A similar study with experimental animals after producing liver injury and experimental diabetes.

The initial work is in progress.

List of Papers Published from the Haffkine Institute during the year 1952.

1. BHARUCHA, Z. K., and MODI, B. G.—The Isolation of Enteric Group of Organisms from Contaminated Blood Samples. A Comparative Study of DEC & BSA Media. (In Press).
2. DUTTA, N. K., ANANTANARAYANAN, K. G.—Release of Histamine from Rat Diaphragm by Cobra Venom. *Nature*, 169 : 1064, 1952.
3. DUTTA, N. K., MEHTA, C. J., & ANANTANARAYANAN, K. G.—Study on the Stability of Vitamins in Oral Preparations. Part I. Effects of Salts of Iron and Copper on Vitamin B₁. *Ind. J. Pharmacy*, 14 : 53-55, 1952.
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7. GANAPATHI, K. & KULKARNI, K. D.—Chemistry of the Thiazoles, Part IV Bromination and Nitration of some mono substituted Thiazoles. (In Press).
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9. GANAPATHI, K. & KULKARNI, K. D.—Chemistry of the Thiazoles, Part VI Chrysean and some of its Derivatives. (In Press).
10. GANAPATHI, K. & PALANDE, B. N.—Chemotherapy of Malaria, Part VIII Synthesis of Uracils, Thiouracils, Pteridines and Thiopteridines. (In Press).
1. LAHIRI, D. C.—Purification and Properties of the Immunologically Synergic Fraction Separated Out of Impurities of the Crude Veal-Infusion Proteose-Peptide Diphtheria Toxoid. *Ind. J. Med. Res.*, 40 265-275, 1952.
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16. PAVRI, K. M.—Infantile Diarrheas, Part I. A Review of Causative Agents.. Ind. J. Child Hlth., 1: 418-434, 1952.
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19. SIDDONS, L. B.—A Preliminary note on the detection of an anti-malarial compound by the mosquito test. (In Press).
20. SOKHEY, S. S. & HABBU, M. K.—The Lag Phase in the Growth Curve of *Pasteurella pestis*. Bull. W. H. O. 6: 65-72, 1952.

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