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REPORT
OF
THE HAFFKINE INSTITUTE

For the Year 1950

BY

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BOMBAY

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1950.

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1950.

Part I.

• GENERAL

I. STAFF

Director

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

Assistant Directors

Dr. D. W. Soman, M.B.B.S., B.Hy. (Bom.), D.P.H. (R.C.P.S.), D.T.M. & H. (Lond.), B.M.S. (I), I/c Department of Clinical Pathology.
(on deputation in U.S.A. from 3rd April 1950).

Dr. A. K. Hazra, M.B.B.S. (Bom.), D.T.M. (Cal.), B.M.S. (I), officiating as Assistant Director I/c Department of Clinical Pathology in addition to his duties, from 3rd April 1950.

Dr. D. C. Lahiri, M.B. (Cal.), D.T.M. & H. (Eng.), D.Bact. (Lond.), 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 Virus Diseases including Rabies.

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

II. GENERAL REVIEW

The Institute continued to function during the year as (a) the centre for the manufacture of plague vaccine for the whole of India and of other prophylactic vaccines for the State; (b) the centre for the manufacture of antirabic vaccine for the Bombay State and adjoining areas and treatment centre for the Bombay City; (c) centre for the manufacture of sulphathiazole, vitamin tablets, casein hydrolysate and other parenteral solutions for the use of Government Hospitals and Institutions in the State; (d) as analytical laboratory for enforcement of Drug Act, 1940, and as (e) the Provincial Bacteriological Laboratory for the Bombay State. Researches on problems bearing on the various activities of the Institute were also carried out, some of them with generous assistance of the Indian Council of Medical Research.

The demand for plague and cholera vaccines was low as in the previous year, viz., 9 million millilitres and 6 million millilitres respectively. The lower demand is happily due to lessened incidence of disease and not to any falling off in the popularity of the vaccines as a prophylactic measure.

D.D.T. Campaign launched in this State for the control of malaria, has, not only brought about a considerable reduction in the incidence of malaria, but a fall in the incidence of diseases, like plague and intestinal diseases like dysentery and cholera has also been noticed. To a lesser extent this also holds good in case of some other states which have adopted DDT applications for plague prevention purposes. The sum total effect of these operations showed that there has been a fall in the incidence of these two diseases in the last two years.

Work directed to bring about improvement in prophylactic vaccines as also in the treatment of human plague infection was continued. In view of the occasional complaints received about the severe reaction resulting from the agar T.A.B. Vaccine, effort was made to prepare a vaccine in liquid casein hydrolysate and this attempt seems to have yielded results. A vaccine which is somewhat superior in potency to the agar vaccine has been prepared and in experimental animals this has not been found to be more toxic than the agar vaccine. In view of the experience gained with the casein hydrolysate cholera and plague vaccines, it is expected that this vaccine would give less reactions in human subjects and experiments to test this are in progress.

Another antibiotic—Terramycin—has been found to exert a curative action in experimental plague infection. Its action is comparable to that of Chloromycetin inasmuch as a much larger dose (336 mgm) than that of aureomycin is required to be given to mice to get the same results. Clinical trials are to be carried out before recommending their use in human infection.

Again the demands for the use of antitoxins and sera continued to be much in excess of what the Institute could produce. The demand came not only from this State but also from Defence Services and other States. As the Department is organised primarily to meet the needs of this State and its productive capacity is thus limited many of the extra-provincial demands remained unsatisfied. Another difficulty arose as a result of outbreak of epidemic lymphangitis amongst the horses, which resulted in curtailment of production. To get over this difficulty and to prevent complete stoppage of serum production, a stud farm was requisitioned near Poona to house additional horses and put them through preliminary stages of immunization; by the time the disinfection of the stables of the Institute is completed, these horses would be ready for final immunization and would thereby prevent long gap in the productive activity of this Department, and supply position would not be materially affected. Steps to acquire this farm on a permanent basis have been taken up so that an additional unit for the production of antitoxins can be developed and the great difficulty experienced by the Defence Services and other States in getting adequate supplies of requisite quantity of antitoxins and sera would be greatly minimised.

The Department of Chemotherapy continued the regular manufacture and sale of sulphathiazole tablets and injectibles. In view of the rising costs of the raw materials, equipment, etc., it became necessary to increase the sale price of sulphathiazole, and fix it at Rs. 35 per 1,000 tablets. A new process for the manufacture of sulphathiazole has been developed and patented and it is expected to give at least 50 per cent. more yield with the resultant reduction in the cost of manufacture.

The supply of Hydnoceol Oil injectible and "Sulphetrone injectibles" to hospitals and clinics in the State for the treatment of leprosy patients was continued and a total supply of 1,53,800 ml. and 43,500 ml. respectively was made. Experiments carried out with a view to explore the possibility of manufacture of Sulphetrone in the Institute have yielded encouraging results and it is felt that with adequate staff and equipment, this Institute would be able to manufacture enough drug to meet the requirements of this State. Investigations in the manufacture of Vitamin B₁ have been undertaken and synthesis of compounds of potential value in the treatment of tuberculosis, malaria and intestinal infections has been continued. Methods have also been standardised for the testing of activity of antimalarial compounds in mice against *P. berghei* infection, which would serve as a rapid method for screening antimalarial compounds for final test. Experiments calculated to bring about improvements in the available method of synthesis of para-aminosalicylic acid (Pas) have also been successfully carried out.

The Department of Pharmacology gave increasing assistance to the Drugs Controller of this State as well as of other States in the enforcement of the Drugs Act, 1940, by examining a large number of samples sent by them. It also afforded greater facilities to the commercial firms to have their products tested and the results of their own analytical laboratories confirmed. Assistance was also given to small firms who could not maintain their own analysts. The number of samples tested for the Drugs Controllers rose to 467 as compared to 261 in the previous year. Similarly, the number of samples tested for the commercial firms was 214 as compared to 69 in the previous year. Although some improvement was noticed in the quality of the drugs available in the Indian market, the number of samples that did not come up to the standard or did not conform to the specifications of the manufacturers were still high, viz. 39 per cent. of the total. In addition to carrying out routine work, the Department has carried on research on antihistamine drugs.

The work of the Department of Entomology suffered long for want of an Entomologist, with the result that some problems of importance could not be investigated. The Institute has now, however, been able to get an experienced Entomologist in Dr. Deoras and it would now be possible to start investigations into those problems.

The demand made on the Department of Virus Diseases for the rabies vaccine continued to be on the increase; and in spite of having three other centres in the City for giving antirabic treatment, the attendance at the Outpatient Department of this Institute remained high. The Department continued to serve as an inoculation Centre for Yellow Fever and other vaccines for international travellers. The necessity of developing the work of the virus department has long been felt and with this object in view Dr. D. W. Soman was deputed to U. S. A. to undergo general and specialised training in virus diseases. On his return it is proposed to develop the Department of Virus Diseases for further work in virus diseases, particularly with regard to influenza, typhus and rabies.

The Department of Clinical Pathology has continued the good work of giving diagnostic aid to various hospitals in the City and free diagnostic aid in the case of infectious diseases and has also started work

recently as a reference laboratory for the venereal diseases. The Blood Bank has continued the supply of dried plasma to the hospitals and medical practitioners in the City and in the mofussil, but the supply of blood for this purpose is still coming primarily from the jails and not from voluntary donors. The Desivac Plant obtained by the Government from the military authorities has now started operation and with this it is proposed to start lyophilisation of other antitoxins and sera which were not so far done, except in the case of Polyvalent Antivenene. This would practically eliminate any loss at present resulting from the non-use of liquid sera during their potency period, as the life of the lyophilised sera is expected to extend to a period of about 10 years.

The demand for the parenteral solutions like glucose saline, casein hydrolysate etc. is increasing year by year and since the inception of the section in 1947 it has increased almost five-fold bringing additional revenue. Any further expansion of demands would necessitate the increase in the present staff and equipment to meet those demands.

Every help is being given to facilitate the work of the new Department of Nutrition which is located in the building of this Institute, but is now administratively separated from it.

III. DEPARTMENT OF VACCINES.

During the year under review, the demand for the various vaccines was the same as during the last year, but lower than the two previous years. Thus while 40 million millilitres of vaccines were issued in 1947 and 23 million millilitres in 1948, the demand for vaccines during 1949 and 1950 was for 15 million millilitres each year. The D. D. T. Campaigns launched in this State for the control of malaria and D. D. T. applications carried out during epidemic periods in other States are in no small measure responsible for the lesser incidence of plague and cholera during the last two years with the resultant less demand for vaccines. The demands for vaccines were received from different parts of India.

A. Plague Vaccine.

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

Months	Quantity in millilitres
January	8,28,240
February	25,80,982
March	8,56,166
April	5,78,680
May	2,37,987
June	15,772
July	47,395
August	2,48,055
September	1,60,230
October	4,62,365
November	12,43,150
December	18,65,380
Total	91,24,402

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

Years.	Millilitres.
1898-1901	86,01,123
1902-1906	103,66,611
1907-1911	107,52,786
1912-1916	137,02,098
1917-1921	200,03,682
1922-1926	216,07,908
1927-1931	177,87,729
1932-1936	199,09,176
1937-1941	200,78,811
1942-1946	445,28,208
1947-1950 (4 years)	578,43,873
Total	2,451,82,005

The Plague Vaccine is issued to India and other countries from this Institute. The demands within and without India met during the year under report are shown below :—

Quantities in millilitres

<i>India.</i>	<i>1950.</i>
Bombay State	4,79,941
Madras State	2,12,005
West Bengal	800
Bihar	7,02,400
Uttar Pradesh	33,28,540
Vindhya Pradesh	83,250
Madhya Pradesh	16,86,900
East Punjab	90,410
Delhi	2,336
Assam	200
Hyderabad (Deccan)	20,20,536
Mysore	10,520
Saurashtra	24
Rajasthan	6,420
Total	8,624,282

Foreign Countries.

Malaya	120
Burma	5,00,000
Total	5,00,120

B. Cholera Vaccine.

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

Months.			Quantity in millilitres.
January	...	...	47,120
February	...	...	4,51,938
March	...	...	56,279
April	...	...	2,28,304
May	...	...	1,89,564
June	...	...	2,98,520
July	...	...	16,84,763
August	...	...	16,48,988
September	...	...	5,02,007
October	...	...	8,82,332
November	...	...	2,59,211
December	...	...	55,296
Total			6,304,322

C. T. A. B. Vaccine.

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

Months.			Quantity in millilitres.
January	...	...	14,644
February	...	...	23,356
March	...	...	13,289
April	...	...	28,278
May	...	...	7,196
June	...	...	34,924
July	...	...	48,397
August	...	...	34,208
September	...	...	37,727
October	...	...	25,138
November	...	...	29,381
December	...	...	15,723
Total			3,12,261

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 clinical pathology department and other routine work carried out at the Institute.
- (3) The manufacture of intravenous fluids.

1. Casein Hydrolysate medium as standardized at this Institute is being manufactured on a large scale for the production of plague and cholera vaccines. During the year, 13,000 flasks (1000 cc. in each) of plague medium and 23,000 flasks (500 cc. in each) of cholera medium were prepared. For the preparation of T. A. B. Vaccine, 1835 bottles (200 cc. in each) of Agar were prepared in acid digest broth.

2. Different types of media were prepared for the routine work of the various departments of the Institute. For testing the sterility of vaccines, toxins, antitoxins and fluids used for intravenous administration, the aerobic and anaerobic media were prepared on a large scale. Thus during the year, 348,632 bottles (80 cc. in each) of aerobic medium and 348,632 bottles of anaerobic medium were prepared, in addition to a number of other media.

3. The manufacture of intravenous fluids was started in the year 1946, with a view to make them available to the poor patients at cheap rates. These products have now become popular and the demand for them is increasing every year. If the demand increases still further, the present staff will be inadequate to carry out the work. The following table gives the comparative figures for the last four years.

Sale of Glucose Saline, etc., during 1950.

S. No.	Name of Product.	No. of bottles sold.	Amount.
			Rs.
1.	Glucose Saline ...	26,129	52,258
2.	5 per cent. Glucose Solution ...	4,869	9,738
3.	25 per cent. Glucose Solution, 100 cc.	3,198	1,599
4.	25 per cent. Glucose Solution, 500 cc.	1,106	2,765
5.	Protein Hydrolysate ...	5,370	10,740
6.	Normal Saline ...	1,532	3,064
7.	Hypertonic Saline ...	3,594	7,188
8.	Sodium Sulphate, Isotonic ...	553	1,106
9.	Distilled Water ...	760	380
	Total ...	47,111	88,838

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

			Rs.
1947	...	10,250	15,986
1948	...	15,995	20,781
1949	...	31,718	46,542

IV. DEPARTMENT OF ANTITOXINS AND SERA.

During the year under review the routine production of antitoxins and sera suffered greatly owing to the continuance of the Epizootic Lymphangitis amongst the horses. The epizootic showed no signs of abatement in spite of all attempts to control it since its outbreak in 1947. In this connection help was sought from the veterinary authorities both Civil and Military under whose instructions it was finally decided to destroy all the horses at the Institute by the end of December, and subsequently to disinfect the stables very thoroughly during the summer months of 1951.

However, the outstanding commitments regarding supply of antitoxins and sera made it imperative for us to continue the production anyhow. With the approval of the Government, therefore, a new farm was hired temporarily at Pimpri to house new horses, and preliminary immunization of these horses started, so that when the stables at the Institute were disinfected and ready to receive new horses, these preliminary immunized horses could be transported and full scale production could be taken up immediately.

Thus the farm at Pimpri near Poona now houses about 100 horses received from different Remount Depots. Efforts are being made to arrange the production of antisera so that essential supplies can be maintained. It would, however, be very difficult to meet each and every requirement of antitoxins and sera received from various quarters.

The epizootic demanded the most serious and continuous attention of the department, with the result that much attention could not be paid to improvements in methods and techniques of the preparation of the sera.

As usual, the demands far exceeded our limit of production. Some of these demands came from the fighting services and had to be met. Unless, however, the limit of present production is extended by setting up of additional units, the department will be compelled to refuse supplying many of the important demands. Maintenance of the very high standards of production and the very low prices, is certain to make even the present exorbitant demands to multiply many times. However, it might be possible in future to extend the activities of the department to meet this expected situation.

1. *Horses*.—124 horses were used during the year for the production of different sera. Besides a number of preliminarily immunized horses varying between 25 to 30 were kept in readiness. The horses were grouped as follows:—

Antitoxins.		Number of horses used.
1. Tetanus Antitoxin	...	68
2. Diphtheria Antitoxin	...	27
3. Anti-snake-venom Serum	...	17
4. Welchii Antitoxin	...	6
4. Anti-dysentery Serum	...	2
5. Anti-human Serum	...	2
6. Normal Horse Serum	...	2
		124

2. *Preparation of Toxins and Toxoids*.—During the year, only tetanus and diphtheria toxins and toxoids were prepared for immunization and hyper-immunization of horses. The reserve stocks of the other toxins and toxoids sufficed during the year. Some portions of both the toxoids were specially reserved for human use.

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

Types of Toxins and Toxoids	Quantity in litres
1. Tetanus	... 479.7
2. Diphtheria	... 222

3. *Preparation of Antitoxins and Sera*.—The total volume of fully processed antitoxins and sera which was made available for human use during the year amounted to 816,400 ml.

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

I. Concentrated Sera.

No.	Name.	Amount of concentrated serum in ml.
1.	Tetanus antitoxin	... 445,800
2.	Diphtheria antitoxin	... 149,100
3.	Gas-Gangrene Antitoxin (Welchii)	... 45,800
4.	Polyvalent Anti-snake Venom Serum	... 79,900
Total		... 720,600

II. Natural Sera.

No.	Name	Volume in ml.
1.	Anti-dysentery Serum	... 37,700
2.	Normal Horse Serum	... 45,100
3.	Anti-human Horse Serum	... 13,000
Total		... 95,800

4. *Lyophilization of Sera.*—Only the polyvalent anti-snake-venom serum was issued in lyophilised form. Arrangements are being made to extend this process to other sera. The polyvalent anti-snake-venom serum amounting to 46,110 ml. was lyophilised during the year.

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

	Quantities supplied in millilitres
1. Tetanus Antitoxin—	
(a) Prophylactic 3,000 I. U. in 2 cc. ...	226,492
(b) Curative 10,000 I. U. in 5 cc. ...	18,435
(c) Curative 20,000 I. U. in 10 cc. ...	111,840
(d) Highly concentrated 20,000 I. U. in 4 cc. ...	45,552
2. Diphtheria Antitoxins—	
(a) Prophylactic 1,000 I. U. in 2 cc. ...	802
(b) Curative 5,000 I. U. in 5 cc. ...	3,740
(c) Curative 10,000 I. U. in 10 cc. ...	48,520
(d) Highly concentrated 10,000 I. U. in 5 cc. ...	15,390
3. Gas-Gangrene Antitoxin—	
(a) 3,000 I. U. Welchii Antitoxin in 10 cc. ...	69,630
(b) 9,000 I. U. Welchii 4,500 I. U. septique and 9,000 I. U. Oedematiens Antitoxins 15 cc. ...	4,380
4. Anti-Plague Serum 20 cc. ...	240
5. Lyophilised Polyvalent Anti-snake Venom Serum 10 cc. ...	46,110
6. Anti-Dysentery Serum—	
(a) Natural 20 cc. ...	1,760
(b) Concentrated 4,000 I. U. in 5 cc. ...	310
7. Tetanus Toxoid—	
(a) 10 cc. ...	910
(b) 5 cc. ...	215
(c) 1 cc. ...	523
8. Diphtheria Toxoid—	
(a) 5 cc. ...	170
(b) 1 cc. ...	348
9. Lyophilised Schick Test Toxin for 25 Tests ...	2 ampoules.
10. Normal Horse Serum in 10 cc. ...	1,720

V. DEPARTMENT OF ENTOMOLOGY.

This department renders free technical assistance to Health Authorities of all parts of the Indian Union in plague work and allied subjects. Several enquiries about rats, fleas and other insects and their control were attended to.

Samples of rat traps sent by different authorities were examined and expert opinions were offered.

The Sanitary Inspectors were given a short course of lectures and demonstrations on plague, rat examination and rat destruction.

Two courses of training in Plague and Anti-plague Measures were held, one in March and the other in September 1950 for the benefit of Medical Officers, and Sanitary Inspectors of the State of Bombay. In all four Medical Officers and thirty-eight Sanitary Inspectors attended these courses.

In addition, several samples of flea collections were received from the Plague Organisation, Public Health Department, Poona, for detection of plague infection in these. In all twenty-three flea-pool inoculation tests were carried out, all proving negative to plague.

A. Examination of Bombay City Rats.

A permanent organisation maintained by the Bombay Municipality, sends to the Institute about 3,000 rodents every day. These include both dead and trapped rats. These rats are classified according to the species and the dead ones are dissected and examined for any evidence of plague infection. In suspicious cases, smears, cultures and animal inoculation tests are carried out. This examination is aimed at providing the Municipal authorities the first signal of presence of plague infection or epizootic in any part of the city with a view to control the spread of the infection. Besides, this work furnishes valuable data on the subject of plague epidemiology.

Tables detailing the total number of rats received and the number examined are given below. During this year, no plague infected rats were detected.

TABLE I—*Showing rats received at the Institute from the Bombay Municipality during 1950.*

Month	Alive	Dead	Total
January	88,226	4,248	92,474
February	80,491	3,691	84,182
March	72,264	3,469	75,733
April	83,093	4,213	87,306
May	80,763	3,481	84,244
June	78,714	3,016	81,730
July	89,319	1,460	90,779
August	91,909	1,302	93,211
September	85,558	1,546	87,104
October	90,529	2,528	93,057
November	89,416	1,443	90,859
December	88,054	1,338	89,392
Total	10,18,336	31,735	10,50,071

TABLE II—*Showing dead rats examined for plague during 1950.*

Month	Dead rats examined	Found plague-infected
January	1,018	Nil
February	778	Nil
March	618	Nil
April	1,010	Nil
May	594	Nil
June	861	Nil
July	664	Nil
August	778	Nil
September	651	Nil
October	1,385	Nil
November	1,356	Nil
December	1,190	Nil
Total	10,903*	Nil

* In addition to dead rats, 461 live rats from the plague infected localities were examined during the year. The total number of live and dead rats examined is 11,804.

TABLES III, IV AND V.

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TABLE III—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> ...	14,165	23.3	14,590	22.4	11,132	21.0	14,323	21.8	15,881	22.8	14,064	21.6
<i>Rattus norvegicus</i> ...	10,896	17.9	10,564	16.2	8,450	15.9	11,185	17.0	12,981	18.7	12,423	19.0
<i>Gunomys kok</i> ...	26,343	43.4	29,893	45.9	24,650	46.5	30,143	45.8	23,683	42.7	27,055	41.8
<i>Bandicota malabarica</i> ...	469	0.8	494	0.8	460	0.9	287	0.4	225	0.3	359	0.5
<i>Mus dubius</i> ...	3,511	5.8	3,368	5.2	3,166	6.2	3,799	5.8	4,050	5.8	4,400	6.7
<i>Suncus caeruleus</i> ...	5,318	8.8	6,147	9.5	5,063	9.5	6,043	9.2	6,737	9.7	7,239	11.0
Total ...	60,712	100.0	65,186	100.0	58,021	100.0	65,781	100.0	69,557	100.0	66,539	100.0

Rats included under this head though reported to have been caught alive, were received at the Institute rats in Table I, because rats received on Sundays and Holidays before 1st August 1930, were not classified.

TABLE IV—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> ...	321	10.0	273	8.6	182	6.9	372	10.3	234	7.4	337	12.8
<i>Rattus norvegicus</i> ...	263	8.2	183	5.8	125	4.8	200	5.5	149	4.7	168	6.4
<i>Gunomys kok</i> ...	421	13.1	318	10.0	508	11.8	401	11.2	310	6.7	343	13.1
<i>Bandicota malabarica</i> ...	8	0.3	1	0.0	3	0.1	3	0.1	1	0.0	...	...
<i>Mus dubius</i> ...	3	0.0	3	0.0	...	...	19	0.5	...	...	2	0.1
<i>Suncus caeruleus</i> ...	24	0.7	35	1.0	3	0.1	29	0.8	...	...	31	1.2
<i>Putrid</i> ...	2,183	67.7	2,385	74.5	1,935	76.3	2,590	71.5	2,561	81.2	1,743	66.4
Total ...	3,223	100.0	3,198	100.0	2,615	100.0	3,517	100.0	3,158	100.0	2,624	100.0

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

of rats trapped alive by Municipal Corporation, Bombay, during 1950.

July.		August.		September.		October.		November.		December.		Whole year.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
15,890	22.5	21,776	20.7	20,662	24.1	22,767	25.2	26,414	29.6	22,238	25.2	2,13,914	23.9
12,611	17.9	17,044	18.5	16,221	19.0	15,905	17.6	14,304	16.0	14,850	16.9	1,67,493	17.6
23,414	40.2	34,580	37.8	31,369	36.7	31,059	34.3	27,314	30.6	31,976	36.3	3,52,493	37.3
642	0.9	611	0.6	677	0.8	579	0.6	488	0.5	861	1.0	6,132	0.7
4,695	6.6	7,316	8.0	6,601	7.7	8,114	9.0	9,417	10.5	8,391	9.5	66,959	7.3
8,404	11.9	10,622	11.6	10,038	11.7	12,055	13.3	11,449	12.8	9,798	11.1	98,883	11.0
70,656	100.0	81,609	100.0	85,668	100.0	90,323	100.0	89,416	100.0	88,054	100.0	8,95,818	100.0

dead in much greater number than the rats received alive. These totals do not agree with the totals of live

species of rats found dead in the City of Bombay during 1950.

July.		August.		September.		October.		November.		December.		Whole year.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
209	19.4	298	22.9	191	12.4	425	16.8	421	29.2	289	21.5	3,552	12.8
155	14.3	207	15.9	192	12.4	338	13.4	317	21.9	352	26.3	2,649	9.6
294	27.2	255	19.6	268	17.3	614	24.3	612	42.4	546	41.0	4,596	16.4
...	...	...	...	...	...	1	0.0	3	0.2	1	0.1	21	0.1
6	0.6	18	1.4	...	...	7	0.3	3	0.2	...	...	61	0.2
...	...	17	1.3	7	0.5	8	0.3	5	0.4	2	0.1	166	0.6
416	38.5	507	38.9	583	37.4	1,135	44.9	82	5.7	146	10.9	15,633	50.1
1,080	100.0	1,302	100.0	1,646	100.0	2,528	100.0	1,443	100.0	1,338	100.0	27,971	100.0

received on Sundays and Holidays were not classified before 1st August 1950.

B. Examination of Rats from the

These rats were received along with those of the other wards of the totals given in previous tables. A separate record of these rats, giving the instance of the Director-General of Health Services.

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

Species	January		February		March		April		May		June	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus rufescens</i> ...	511	56.8	867	44.4	405	57.5	279	43.8	339	44.1	329	44.4
<i>Rattus norvegicus</i> ...	79	8.8	81	9.8	60	8.5	114	17.9	82	10.7	139	19.3
<i>Gunomys kok</i> ...	226	25.1	201	24.3	151	21.5	178	28.0	247	32.1	153	21.3
<i>Bandicota malabarica</i> ...	4	0.4	1	0.1	2	0.3	...	...	...	...	2	0.3
<i>Mus dubius</i> ...	35	3.9	138	16.7	33	4.7	27	4.2	23	3.0	26	3.6
<i>Synomys coreleus</i> ...	45	5.0	59	4.4	33	7.5	59	8.1	78	10.1	50	11.1
Total ...	900	100.0	827	100.0	704	100.0	637	100.0	769	100.0	730	100.0

Rats received on Sundays and Holidays before

Bombay Port Trust Docks.

Bombay Municipality, and have been already accounted for in the monthly number and percentage of the different species, is also maintained at the

of rats caught alive from the Bombay Port Trust area in 1950.

July.		August.		September.		October.		November.		December.		Whole year.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
281	37.9	509	52.6	404	44.6	505	52.2	532	54.8	420	44.5	4,872	48.4
133	17.9	174	18.0	189	22.0	161	16.6	155	15.9	198	21.0	1,575	15.7
221	30.0	166	17.2	100	21.0	188	19.4	161	16.6	211	22.3	2,293	22.8
3	0.4	9	0.9	...	...	5	0.5	3	0.3	8	0.8	37	0.4
19	2.6	41	4.2	52	5.7	65	6.7	55	5.7	41	4.3	555	5.5
83	11.2	69	7.1	61	6.7	44	4.6	65	6.7	67	7.1	723	7.2
740	100.0	968	100.0	906	100.0	968	100.0	971	100.0	945	100.0	10,055	100.0

1st August 1950, were not classified.

C. Examination of rats from steamers and other sources.

During the year 1950, three dead rats and twenty-nine smears of dead rats were received from different towns of the Bombay State. None of them was found to be plague infected. In the same year, 450 killed rats from steamers were received* from the Health Officer of the Port of Bombay and of these 415 belonged to the species *Rattus rattus rufescens*, 18 *Rattus norvegicus*, 8 *Gunomys kok*, 8 *Mus dubius* and 1 *Suncus caeruleus*. None of them was found plague infected.

VI. DEPARTMENT OF CLINICAL PATHOLOGY.

A. Bacteriological Section.

This section examined clinical material from various Government hospitals and dispensaries in the city and the mofussil places in the State. In addition, private medical practitioners contributed a good number of samples for diagnostic work. Free diagnostic aid was given to all practitioners in infectious diseases such as diphtheria, dysentery, cerebro-spinal fever, enteric fever, typhus fevers, cholera, plague, etc. The typhoid scheme sanctioned by Government in 1947, continued its operation during the year 1950 and the Institute doctor attended 856 calls from private medical practitioners. The scheme has become popular and a large number of medical practitioners are taking advantage of it. It also helps the city health authorities to get early notification of this disease and enables them to take preventive measures in time.

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

No.	Nature of Examination.	No. of samples examined.	No. of positive samples.
1	Wassermann Test	4,977	1,488
2	Kahn Test	1,688	322
3	Widal Test	2,354	758
4	Blood cultures for enteric group of organisms	2,747	427
5	Agglutination test for typhus fever	2,796	183
6	Agglutination test for Brucellosis	2,869	10
7	Material for T.B. by smear, culture and animal inoculation test	521	105
8	Throat swabs for diphtheria	120	39
9	Stools for microscopic examination	958	292
10	Stools for culture (including rectal swabs)	1,855	181
11	Blood films for malaria	676	27
12	Material for plague	10	1
13	Friedman test and rat test for pregnancy	318	127
14	Urine for cultural examination	172	42
15	Urine for chemical and microscopic examination	665	
16	C.S.F.—routine and culture	295	
17	Blood counts	872	
18	Material for detection of gonococci	105	
19	Material from medico-legal cases	14	
20	Rideal-Walker Test	54	
21	Autogenous vaccines	57	
22	Semen examination	56	
23	Agglutination test for Leptospirosis	56	5

The city had an epidemic of cholera during the months of July, August and September, during which period 932 samples were examined and 83 strains of *V. cholera* were isolated. 41 strains of dysentery organisms, 1 of *B. typhosus* and 3 of salmonella group were isolated, on routine cultural examination of stools. Out of 41 strains of dysentery organisms that were isolated, 28 belonged to *B. flexner* type, 3 to *B. Shiga* type, 6 to *B. schmitz* type, 1 to *B. sonne* type and 3 to "Atypical dysentery" group.

This section was also engaged in the maintenance of various species of poisonous snakes for the purpose of extraction of venom. This venom is used for the preparation of polyvalent antivenene in this and other Institutions. The venom is freeze-dried and maintained in vacuum sealed ampoules at refrigeration temperature. The quantities of different venoms collected and supplied during the year under report are given below :—

Cobra venom in gms.		R.V. venom in gms.		Krait venom in gms.		Echis venom in gms.	
Collected.	Supplied from stock.	Collected.	Supplied from stock.	Collected.	Supplied from stock.	Collected.	Supplied from stock.
19557	17697	18935	18756	1887	4062	Nil.	4123

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

No.	Reagents.	Amount supplied.		
1	Blood grouping sera	...	...	4225 ml.
2	High titre sera	...	...	341 ml.
3	Killed suspensions for Widal and Weil-Felix test...	...	...	101910 ml.
4	Kahn antigen	...	...	1437 ml.
5	Ambocaptor	...	...	49 ml.
6	W.R. antigen	...	...	240 ml.
7	R.V. venom solutions 1 : 1,000	...	...	293 ml.
	1 : 3,000	...	...	252 ml.
	1 : 10,000	...	...	254 ml.
8	Cobra Venom Solutions 5 M.U.	...	...	164 ml.
	10 "	...	...	115 ml.
	15 "	...	...	71 ml.
	20 "	...	...	25 ml.
	25 "	...	...	26 ml.
	Complete box of graduated doses	...	...	146 boxes.

B. Section of Public Health Chemistry and Bacteriology.

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

The following table gives a summary of the various examinations carried out during the year 1950 :—

Sample examined.	Nature of examination.	No. of samples examined.
Water	... Fitness for drinking purposes	... 74
Water (Aerated)	... Fitness for drinking purposes	... 1
Water	... Fitness for sanitary and industrial purposes	... 24
Milk	... Bacteriological grading	... 1
Marmite	... Bacteriological examination	... 1
Sweet potato flour	... } Bacteriological examination	... 1

C. Histo-Pathology Section.

During the year under review, 944 pathological specimens, received from different hospitals in this State and a few from outside the State of Bombay were examined. These include brains from suspected rabid animals, neoplasms, gynaecological conditions, tuberculosis of different organs and some rare and interesting pathological specimens. In addition, 112 specimens from experimental animals were examined to study the morbid histological changes. These were mostly from the Experimental Pathology Section which carries out histological examination of tissues for Research Inquiries conducted at the Institute. In all 1,056, specimens were examined in the department during the year.

The table below gives classification of the morbid histological condition of the tissues received and examined at the Institute during the years 1949 and 1950 :—

Classification of pathological specimens received for examination.

	1949	1950
(1) Tissues found to be normal	42	55
(2) Tissues unfit for examination	66	88
(3) Brain tissue for Negri Bodies	118	93
(4) Miscellaneous Pathological Conditions	457	505
(5) Tuberculosis	39	47
(6) Non-malignant neoplasms	114	72
(7) Malignant tumours	96	84
Total	932	944

Detailed classification of pathological conditions of interest :—

I. NEOPLASMS :

(a) *Malignant :—*

(1) Carcinoma Primary Adenocarcinoma	...	8
Carcinoma Simplex	...	25
Epidermoid carcinoma	...	22
Scirrhus carcinoma	...	1
Squamous-cell carcinoma	...	2
(2) Carcinoma secondary	...	5
(3) Sarcoma :		
Fibrosarcoma	...	6
Melanotic sarcoma	...	1
Sarcoma	...	2
(4) <i>Malignant Lymphoma :</i>		
Hodgkin's sarcoma	...	3
Lymphosarcoma	...	1
(5) Malignant melanoma	...	1
(6) Seminoma	...	4
(7) Haemangio-endothelioma	...	1
(8) Mixed parotid tumour	...	1
(9) Teratoma	...	1

(b) *Non-malignant Neoplasms :—*

Cavernous haemangioma	...	1
Colloid goitre	...	10
Cystadenoma	...	1
Fibroadenoma	...	7
Fibrolipoma	...	1
Fibroma	...	9
Fibromyoma	...	20
Fibrous epulis	...	4
Lipoma	...	4
Melanoma	...	1
Neurofibroma	...	2
Osteochondroma	...	1
Osteoclastoma	...	1
Papillary cystadenoma	...	2
Papilliferous cystadenoma	...	6
Papilloma	...	1
Pseudomucinous cystadenoma	...	1

II. TUBERCULOSIS IN DIFFERENT ORGANS :

Breast	...	3
Female genital tract	...	20
Intestinal tract	...	5
Lymphnodes	...	19

III. UNCOMMON SPECIMENS RECEIVED DURING THE YEAR :

(i) Brenner's tumour of the ovary	...	2
(ii) Splenule	...	1
(iii) Hypernephroma of liver	...	1
(iv) Adenoid cystic Epithelioma	...	1
(v) Rhinosporidiosis	...	4

VII. DEPARTMENT OF PHARMACOLOGY.

Drug Testing.—The progress of this Department with regard to the testing of drugs in meeting the enforcement of the Drugs Act, 1940, has been very satisfactory. Nine hundred and twenty-four samples of drugs, medicinal chemicals, biological products, glasswares, disinfectants, surgical dressings, etc., were examined in this department during the year under review. As compared to the last year, the number of samples tested increased by 182 per cent. Out of the total 924 samples, 508, i.e., 44:1 per cent. came under biological and other special products (Schedules C and C1). Although there was some improvement in the quality of the drugs available in the Indian market yet the percentage of rejected samples was fairly high amounting to about 39 per cent. of the total.

Samples were received from the Drugs Controllers or on their behalf from the States of Bombay, Delhi, Madhya Pradesh, Uttar Pradesh and Orissa. Samples were also received from Bombay Customs and Port Trust and from commercial firms. Besides these, a larger number of our own products and those received from the Government of Bombay (other than mentioned above) were examined here. A condensed statement of the results of analysis of drugs, chemicals, etc., is attached herewith.

Commercial Samples.—Greater facilities have been provided to various manufacturing firms to have their products tested on payment of the scheduled fees. Samples were received practically from all parts of the country. As compared to the preceding year, the number of samples reported rose from 69 to 224, an increase by 324 per cent. This department has not only helped the private firms in raising the standard of their products by repeated testing but also assisted them in confirming the results of their own analytical laboratories. Valuable services were rendered to the small firms who could not afford to maintain analysts of their own.

Rejected Samples.—Out of the 21 samples of Tincture Digitalis examined, 16 failed to conform to the B. P. specifications. Of these again 4 preparations did not contain any active glycoside.

Solutions of Calcium Gluconate have tendency to precipitate unless properly stabilised. Probably at the time of issue from the firms, the solutions remained clear but at a later date the precipitates appeared. This would account for the rejection of a large number of Injectio Calcium Gluconate.

Of the four samples of Emplastrum Belladonnae, in 2 the alkaloidal content was very low.

Half the number of Ephedrine Hydrochloride had a low alkaloidal content. One batch of tablets which from the appearance seemed to be very good, was devoid of any alkaloid.

Menthol is expected to contain not more than 0.05 per cent. of non-volatile matter. The sample examined here contained 98 per cent. of non-volatile matter.

Gripe water is one of the popular medicines amongst our countrymen. Out of the 40 samples examined, 37 did not come up to the standard.

Out of the six samples of Milk of Magnesia tested, only two conformed to the standard quality.

As many as 21 samples of "Genuine" Sandal Wood Oil were tested according to the Indian Pharmacopoeial List Standard. Eighteen samples, i.e., about 86.0 per cent. had to be rejected because of their inferior quality.

Several samples of Penicillin were tested biologically and a few iodimetrically. One of the samples contained only 1,00,000 units per mg., although the declared potency was 5,00,000 units per mg. Two samples of Penicillin were said to have been "synthesized" locally. These samples did not satisfy identification and other tests for penicillin.

Forty-nine samples of B. P. Tinctures other than Tincture Digitalis were examined. These included the commonly used preparations like tinctures of Belladonna, Hyoscyamus, Nux Vomica, Quinine Ammoniata, etc. Twenty-nine of these products did not comply to the required specification, the main reason being they were deficient in alkaloidal content.

Samples of Sulphanilamide, Sulphaguanidine, Sulphathiazole, Sulphadiazine were amongst the "Sulpha" group of drugs examined. Samples labelled as "Sulphathiazole" and "Sulphadiazine" were found to be on examination nothing but Sulphanilamide. This type of misbranding is not infrequent.

Seven out of the eight samples of Easton's Syrup (Syrupus Ferri Phosphatis Cum Quinina Et Strychnina) were rejected being not of standard quality. In some cases, the alkaloidal content was less, in others the iron content was low, and still in others a lot of suspended matter was present.

Preparations containing Vitamins.—One hundred and twenty-nine preparations containing vitamins A, B¹, B², B-Complex, C, D, Folic Acid, and Nicotinic Acid, either singly or in combination with other constituents, were tested during 1950. The products were dispensed as tablets, liquids or injectables. The liquid preparations were rather complex in nature containing vitamins, liver extracts, and other inorganic substances. The number of ingredients in any of these products varied from 6 to 13. It was observed that in samples containing salts of iron, copper or manganese, the potencies of vitamins B¹, B² or C were not infrequently deteriorated. An investigation as to the cause of such deterioration has been undertaken.

Test for the absence of Pyrogen.—Parenteral solutions like Glucose Saline, Distilled Water, Protein Hydrolysate, etc., were tested for the absence of pyrogenic substances in rabbits. Two hundred and five products were examined as against 95 during the preceding year. The number of samples which showed pyrogenic reaction were 4.

Toxicity Tests.—One hundred and seventy-seven medicinal preparations meant for intravenous medication were tested in white mice for determining whether they were free from toxic substances or not. The Minimum Lethal Doses were determined in white mice for several synthetic organic compounds.

Several samples of Lyophilized Human Plasma were tested in "bled" cats to find out their resuscitating property. One sample proved to be unsatisfactory.

Sulphanilamide Paste.—The department prepared 212 pounds of paste containing 10 per cent. sulphanilamide and oleum vitaminatum for distribution to hospitals, charitable institutions, etc.

Research and other activities.—Amongst the methods that were introduced one may refer to the following :—(i) microbiological assay of Vitamin B² (ii) assay of Vitamin D by "Lime Test", (iii) standardisation of female sex hormone on overrectimised mice, (iv) spermicidal test using guineapig's sperm, (v) determination of the local anaesthetic action of drugs on guineapigs, and (vi) assay of histamine on guineapigs ileum.

In spite of the heavy pressure of routine work several problems were taken up for investigation. Abstract of the completed work is given in part II of this report.

Condensed statement of the results of analysis of drugs, medicines, etc., during the year 1950.

Name of the Product	No. examined.	Standard quality.	Not of standard quality.
Acid Carbolic	2	1	1
Acid Citric	2	1	1
Acid Hydrochloric	5	1	4
Acid Sulphuric	4	3	1
Adrenaline Hydrochloride	9	5	4
Aletis Cordial (so-called)	7	2	5
Antiphlogistine	4	1	3
Antiseptics and Disinfectants	5	3	2
Aspirin and similar preparations	13	11	2
Atropine Sulphate	4	3	1
Calcium Gluconate	13	6	7
Calcium (other salts)	5	3	2
Camphor (crude)	1		1
Camphor in oil	3	1	2
Chicken Essence	10		
Chlorodyne	3		3
Contraceptive Jelly	1		1
Digitalis (Tincture)	21	5	16
Digitalis (other preparations)	5	3	2
Dihydro Streptomycin	1		1
Distilled Water	18	16	2
Emetine Hydrochloride	7	3	4
Emp. Belladonna	4	2	2
Ephedrine Hydrochloride	14	7	7
Ether (Anaesthetic & Sulphuric)	5	4	1
Ethylene dichloride	3		3
Extract Ergot liquid	2	1	1
Gammexane	13		
Gauze (absorbent)	3	1	2
Glucose Saline (Parenteral)	75	75	
Glucose Solution (Parenteral)	47	46	1
Gripe Water	40	3	37
Horlicks (so-called)	2	1	1
Indigenous drugs	6	5	1
Insulin	3	2	1
Iodine Preparations	5	1	4
Iron Salts	3	2	1
Liq. Ammonia Acetas Fortis	3	1	2
Liq. other preparations	6	4	2
Mercurochrome	3	1	2
Menthol	1		1
Milk of Magnesia	6	2	4
Morphine or other similar preparations	5	2	3
Oestrone	1	1	
Oil Eucalyptus	8	8	
Oil Groundnut	11	9	2
Oil Olive	2	1	1
Oil Sandalwood	21	3	18
Oil Til	6	2	4

Name of the Product.	No. examined.	Standard quality.	Not of standard quality.
Oil Others	5	3	2
Ointments	7	5	2
Other drugs	80	57	23
Para amino salicylic acid	8	6	2
Patent & proprietary medicines (other than mentioned)	19	12	7
Penicillin (Crystalline)	7	6	1
Penicillin (Synthetic)	2		2
Penicillin (Lozenges)	1		1
Penicillin (Ointment)	2	1	1
Pituitary (posterior lobe extract)	1		1
Plasma Human (Lyophilised)	3	2	1
Potassium Acetate	1	1	
Do. Bromide	3	2	1
Do. Citrate	10	5	5
Do. Chlorate	2	1	1
Do. Nitrate	1		1
Protein Hydrolysate (Parenteral)	24	24	
Quinine Bihydrochloride (injections)	18	13	5
Quinine Ethyl Carbonate	4	3	1
Do. Preparations (others)	4	4	
Do. Sulphate	2	2	
Saccharin	3	2	1
Saline Normal	12	10	2
Do. Hypertonic	6	6	
Santonin	2	1	1
Sodium Chloride	2	1	1
Do. Citrate	3	1	2
Do. Salts (others)	10	7	3
Spirit of Ammonia Aromatics	3	1	2
Do. Chloroform	1		1
Do. Ether Nitrosi	2		2
Strychnine Salts	2	2	
Sulphanilamide & its derivatives	18	10	8
Syrup Easton	8	1	7
Do. Codeine Phosphate	3	1	2
Do. Ferri Phosphate	2	1	1
Do. Others	5	3	2
Tincture Belladonna	10	6	4
Do. Benzoin Co.	1		1
Do. Chinchona	2		2
Do. do. Co.	1	1	
Do. Ephedrin Vulgaria	1		1
Do. Hyoscyamus	6	2	4
Do. Ipecacuanha	3	2	1
Do. Nux Vomica	11	6	5
Do. Opii	1	1	
Do. do. Camphorita	2	1	1
Do. Quinine Ammoniata	5		5
Do. Stramonium	4		4
Do. Strophanthus	2	1	1
Vaccines	9	7	2
Vegetable Essence	5	3	2
<i>Vitamin Preparations :</i>			
Vitamin A	20	9	4
Do. B ₁	21	9	12
Do. B Complex	63	21	42
Do. B ₂	5	3	2
Do. C	9	8	1
Do. D	1	1	
Folic Acid	3	2	1
Nicotinic Acid	7	6	1
Waterbury's Compound (so called)	5	2	3
Total	924	584	356

VIII. DEPARTMENT OF CHEMOTHERAPY.

During the year under review, the Department continued the regular manufacture and sale of sulphathiazole tablets and ampoules. In addition to the supply to various Government, municipal and charitable institutions, the Government permitted the sale of sulphathiazole tablets to private medical practitioners. This policy has resulted in an increased demand for the tablets. In view of the rising costs of the raw materials, equipment etc., it was found necessary to revise the sale price of sulphathiazole, which now has been fixed at Rs. 35 per 1,000 tablets. The additional experience gained in the manufacture of sulphathiazole has enabled the department to make further improvements in the efficiency of the process by rearranging the various equipment and unit processes adequately. As a result of this, the manufacture of this important drug has been rendered more systematic and free from most of the usual inherent troubles. Small scale trials on a new process patented by this department for the manufacture of sulphathiazole (Indian Patent No. 39684, dated 17th May 1948), has yielded very encouraging results and it is hoped that when the large scale trials of this improved process would be taken, the average overall yield of sulphathiazole that would be obtained thereby would be at least $1\frac{1}{2}$ times of that which is being obtained at present. This would bring down the cost of manufacture of sulphathiazole considerably. Further trials are under-way. All the orders for the supply of sulphathiazole tablets during the year have been met.

The manufacture and supply of Hydnocarpus oil adequately processed and rendered suitable for injectible purposes, for the treatment of Leprosy in the various leprosy clinics in the State of Bombay was continued. A total of about 1,53,800 c.cs. of the preparation was manufactured during the year.

Under instructions from the Government manufacture of a sterile injectible preparation "Sulphetrone" was undertaken and about 43,500 c.cs. of this very useful drug was supplied to the various State-aided clinics for the treatment of leprosy. The drug is being supplied as a sterile 50 per cent. solution in double-distilled water, in multiple-dose vials. In virtue of the encouraging results reported with the use of sulphetrone in the treatment of leprosy, experiments have now been undertaken with a view to manufacture this drug at this Institute. Preliminary investigations in this direction have been very encouraging and it is hoped that the provision of adequate staff, equipment, etc., may in the very near future enable us to manufacture sulphetrone and other useful derivatives of 4:4'-diaminodiphenylsulphone in this department to meet adequate demands for this important drug from the various dispensaries and clinics in the State of Bombay.

The manufacture of "Pemon" was continued as usual and about 3,700 lbs. of this antiseptic were manufactured during the year under review. The production of Phenyl Mercuric Nitrate had to be increased due to the additional demands for the same from the various departments of this Institute. About 15,000 g. of phenyl mercuric nitrate were manufactured during the year.

Investigations were undertaken to prepare total opium alkaloid solutions suitable for injectible purposes for the J. J. Group of Hospitals from crude alkaloids supplied by them. A suitable procedure for the purification of the alkaloids so supplied was developed and subsequently about 3,000 ampoules each of 1.0 c.c. and containing total alkaloids equivalent to 1/4 grain of Morphine per c.c., were manufactured and supplied.

Various available methods for the synthesis of para-amino-salicylic acid (PAS), one of the useful chemotherapeutic drugs for the treatment of tuberculosis have been investigated and valuable improvements in the already reported procedures have been effected. One of the methods, involving the use of phenyl-acetic acid as the starting material has been extensively studied and sufficient data has now been collected to justify a large scale trial of the same for the manufacture of para-amino-salicylic acid (PAS.).

The comparative value of the practicability of several methods for the preparation of Nicotinic Acid has been studied. The hitherto little investigated processes for the synthesis of Nicotinic Acid using pyridine itself as the starting material have been particularly investigated with encouraging results.

Investigations in the manufacture of Vitamin B₁ (Thiamine Hydrochloride) have been undertaken and the comparative value of the several methods available for the synthesis of this important vitamin is being studied. The results obtained so far appear to be highly encouraging.

As an extension of the work on the synthesis of quinoline derivatives as anti-malarials, methods for the synthesis of very useful amoebicide, 5-chloro-7-iodo-8-hydroxy-quinoline, sold in the market under various popular brands, particularly as "vioform", "Entero-vioform", have been studied, and optimum conditions for the preparation of the same have been worked out. Further work in this direction is in progress.

The synthesis of compounds of potential value in the treatment of Tuberculosis, Malaria and intestinal infections has been continued and several new compounds have been synthesised. The results of the antibacterial and antimalarial activities of the same by in-vitro and/or in-vivo tests of the same are awaited.

Methods have been standardised for the testing of the antimalarial activity of the compounds synthesised in mice against B. Gerghi infection. This method appears to be a rapid and reliable one for the screening of compounds for their antimalarial activity.

Vitamin Tablet Section.

The section worked mainly on two lines: (1) The production of vitamin A Concentrate from the Shark-liver Oil; (2) The manufacture of various types of Vitamin Tablets and Sulphathiazole Tablets.

The Concentrate Section.—This section processed during the year 4,620 lbs. of Shark-liver Oil. Concentrates of average potency of 2,50,000 I. U. per gm. were obtained.

The Tablet Section.—This section continued the manufacture of the Vitamin Tablets and the Sulphathiazole Tablets as usual. The sale of these tablets was found to have considerably increased during the year under report than during the previous years. In addition to the usual orders from hospitals, dispensaries, and the public, other organisations like the Health Services of the Government of West Bengal and the Ministry of Food (Government of India), also purchased a good number of these Vitamin Tablets from us.

The sale of Sulphathiazole Tablets has increased to a considerable extent since the Government has permitted us to sell these tablets to private and charitable hospitals and dispensaries, and private medical practitioners. We have now enough stocks of these tablets to meet the demands.

The number of tablets prepared during the past two years and the year under report are given below :—

Kind of tablets.	Production during		
	1948	1949	1950
1. Vitamin A-D	7,05,470	57,82,620*	56,69,450
2. Vitamin B-C	7,35,080	57,91,010*	64,36,250
3. Vitamin B ₁	1,73,845	2,44,150	1,29,500
4. Vitamin B ₂	36,900	69,290	57,400
5. Vitamin C	1,99,800	4,11,300	2,96,900
6. Nicotinic Acid	16,320	1,65,450	1,14,800
7. Vitamin B-C No. 3			35,560
8. Sulphathiazole Tablets	1,08,000	8,46,655	11,73,000
	19,75,865	133,10,475	139,12,860

*Out of these approximately 4 million of each were prepared for the Government for free distribution to the famine-stricken area.

During the course of the year, it was noticed that our competitive prices were partly responsible for causing a considerable change in the current market prices of tablets manufactured by local and foreign firms. These firms have brought down their prices of vitamin tablets and sulphathiazole tablets which now are almost equal to ours in many cases.

We feel that the existence of this section has served the laudable purpose of compelling other manufacturers too, to make their articles, so essential for the relief of the suffering millions, available at reasonable prices.

The sale of our vitamin products through Government approved dealers is under active consideration of the Government.

IX. DEPARTMENT OF BIOCHEMISTRY.

The department has done active work during the year under review. The total number of samples received from the Hospitals, private practitioners and the Institute increased to 3,168 as compared to 2,935 samples examined during 1949. This comprises .

Blood examinations	...	...	...	1,767
Urine	...	...	...	227
C. S. F. & other Fluids	...	...	...	631
Milk	...	...	...	392
Special Tests	...	...	...	151
(such as B. M. R. determination, Glucose Tolerance Tests, Gastric and Kidney Function tests).				

It was found necessary to modify some of the analytical procedures that were being followed and to replace others by more satisfactory and up-to-date methods so as to improve the quality of work turned out. The introduction of photo-electric colorimetry for all routine work has been a welcome change and working with the Leitz photoelectric colorimeter satisfactory colorimetric methods have been introduced. We are making use of standard calibration curves for all the color reactions and these are checked off and on by a responsible member of the staff.

It is worthwhile to indicate some of the changes made in the methods that were originally used in the department. However, only a few major ones are considered below.

Blood Urea.—The estimation of urea in blood which can range from 5 mg. per cent. to more than 300 mgs. per cent. is based on the principle of the conversion of the urea into ammonia by the enzyme urease. A convenient extract of this enzyme from Jack bean meat can be obtained by using either 70 per cent. Alcohol or Glycerol. We have preferred the glycerol extract to the original alcoholic extract since the former is stable indefinitely in the cold. After the action of the enzyme, the ammonia liberated is usually determined by distillation or by aeration and it has been the experience of many workers that a considerable loss of ammonia can occur during the process, in the hands of an average technician. So conditions were worked out to simplify the procedure and to determine the ammonia directly with the Nessler reagent. The turbidity usually met within Nesslerisation has been overcome by adjusting the amount of the reagent added and the final concentration of sodium hydroxide. Satisfactory results are obtained for all ranges of blood urea. It should be mentioned here that some of the hospitals have been regularly sending blood samples, containing ammonium oxalate as the anti-coagulant for the estimation of urea and Non-protein Nitrogen. This defect has been brought to the notice of such parties.

Blood Sugar.—The method of Folin and Malmros (1929). using potassium ferricyanide for the estimation of blood glucose was being used in this laboratory for many years. The colour of the prussian blue formed in this procedure is, however, unstable and it is often precipitated before a colour reading can be taken. This is a serious drawback in colorimetry. The copper reduction method employing Somogyi's New copper Reagent (1945) and estimating the reduced copper by the formation of the coloured complex with the modified Nelson's reagent (1946) has

been adopted. The new copper reagent, containing phosphate buffer instead of the bicarbonate-carbonate buffer has been recommended by Somogyi as representing the most favourable condition regarding buffer action stability and sensitivity.

A micro modification of this combination has been standardised for all blood samples containing a range of 40-500mg. per cent. glucose.

Fractionation of albumin and globulins.—It is now recognised that the albumin values obtained by the Howe's sodium sulphate fractionation are always somewhat larger than the albumin values obtained by electrophoresis or by chemical fractionation techniques which have been adjusted to electrophoretic standards. The difference between the Howe albumin value and the "true" albumin value has been shown to be approximately equal to the α -globulin concentration. Since the decrease in albumin is always accompanied by increase in α -globulin, any such clinical changes will escape detection by the use of 23 per cent. sodium sulphate for fractionation of serum proteins. It has been worked out that human sera requires 26-27 per cent. sodium sulphate for the precipitation of all globulin fractions and to give the "true" albumin in solution.

The problem of the removal of the precipitated globulins from the albumin in solution has long been a vexation to the analytical chemist. This has been more or less solved by the excellent and simple technique of Kingsley (1940), utilising ethyl ether for the rapid and clear-cut separation of the globulin fraction. We are now following this technique in preference to the traditional method of incubation and filtration at 31°, with the only change in that we have introduced 27.4 per cent. sodium sulphate instead of the original 23 per cent.

Estimation of total protein and albumin by Kjeldahl digestion.—The colorimetric method of Wu and Ling which had been followed in this laboratory for many years was discontinued for specific reasons. This method is based on the use of Folin's phenol reagent to measure the tyrosin component of the protein molecule and has the only merit of simplicity of manipulation but has many serious objections, some of which are :—

- (1) The stability of the colour attained in the various procedures with Folin's phenol reagent is very limited.
- (2) The colour intensity developed is different for albumin, globulin and the colour factors for human serum proteins are different from those of other animals.
- (3) The colour factors obtained with this reagent are different in certain pathological sera.
- (4) The colour developed is dependent upon the age of the serum sample and decreases with time as the serum stands.

In the light of these facts the use of this method was discontinued in preference to the Kjeldahl method of nitrogen determination for the quantitative estimation of serum proteins. The attendant difficulties of time and the rigorous chemical technique needed have precluded the method from routine use in most clinical laboratories. With modified

Kjeldahl methods very good results may be obtained if the following precautions are observed :—

- (1) Careful and complete acid digestion.
- (2) Accuracy in aliquoting the large dilutions necessary.
- (3) The use of a well-balanced Nessler reagent and the colorimetric determinations made with a good spectrophotometer or a photo electric colorimeter.

Thus combining the salient points from the experience of other workers, an accurate and rapid micro method for the estimation of albumin and the globulin has been worked out for routine analysis of serum proteins. Working with some pathological samples it has been possible to show that the "true" albumin fraction can reach as low a figure as 0.5 g. per cent. in some cases of nephrosis. Figures as low as 0.1 g. per cent. are available from recent literatures using both chemical and electrophoretic analysis.

The Biuret Method.—As an alternate method for protein analysis by Kjeldahl digestion we undertook an investigation of a newer method which has been rather popular of late. The Biuret reaction given by all proteins was first made use of by Kingsley (1939) for the routine analysis of serum proteins. This was followed by a series of modifications of the reagent and the conditions. At a glance this method appears to be a very rapid and accurate one, once the conditions have been standardised. In our hands it has been quite successful, as also reported by others, when applied to normal serum. However, with various pathological serum samples regular and definite discrepancies have been noted between the Biuret value and the Kjeldahl value. This has been attributed to :—

- (1) A change in *N* concentration.
- (2) A change in number and reactivity of biuret linkages
- (3) A change in response to salting out with sodium sulphate.
- (4) Or a combination of (1), (2) and (3).

These changes may be related to alterations in number, kind or structural arrangement of amino acids in the protein molecule or altered content of sugar, lipids, etc., forming part of the protein molecule. Peters and Van Slyke have made reference to the altered chromogenic power of serum proteins in disease in connection with the use of tyrosine, as the standard in the Wu-Ling method. Clinical data have been recently published by Kingsley indicating the discrepancies formed and the possible diagnostic value of the determination of the ratio of biuret *N* to Kjeldahl *N* in serum proteins. We are estimating the serum proteins by both the methods to get some more data on the differences observed and finally decide which method would be more accurate for routine work.

Blood cholesterol.—For the estimation of blood cholesterol, drying of the sample is necessary before extraction with chloroform and the subsequent application of the Liebermann's reaction. Usually this is accomplished by drying an *aliquot* spread on a filter paper, in an oven at

60-70°C for one hour. It is a general experience that in spite of this a small amount of moisture is retained which is extracted by the chloroform and this usually interferes with the subsequent colour development. We have found that the sample can be extracted directly without oven-drying by introducing it in between two layers of anhydrous magnesium sulphate. This technique has considerably lessened the time of estimation in addition to giving more accurate and reproducible values.

17-Keto Steroids in Urine.—Since occasional enquiries were made regarding the estimation of 17-Ketosteroids in urine, we have made a preliminary survey of the two methods described in literature. The vigorous experimental conditions involved has limited its scope for routine work. Of the two, the method of Pincus using antimony trichloride has been found to be more encouraging when applied to the crude urine extract. With our limited experience we find that the Zimmermann method gives slightly higher values when compared to the Pincus method. The difference becomes particularly significant when dealing with urine samples of children who are known to excrete very small amounts. So far we have been expressing the values in terms of androsterone as standard. Purification of the crude 17-ketosteroids by the Girard's Reagent before colorimetric estimation will be undertaken shortly.

In addition to these extra routine investigations research work has been planned on some aspects of biological acetylation, biochemical investigation of lathyrism and the clinical investigations of pathological samples by paper chromatography.

X. DEPARTMENT OF VIRUS DISEASES INCLUDING RABIES.

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

(a) *Anti-rabic Treatment Centre at this Institute.*—During the year under report 5,473 persons applied for anti-rabic treatment; of these 4,934 completed the full course of treatment, 539 were "advice cases" In cases where there was a doubt about the condition of the dog inflicting the bite and the dog was available for observation the patients were given two injections and were advised to keep the dog under observation for 10 days. At the end of the period of observation, if the dog was healthy, no further treatment was given and the case was classified as an "advice case".

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

TABLE I.—*Details of treatment carried out at the Treatment Centre of the Institute and the outstation centres in 1946-47, 1948-49, 1949-50.*

Details.	1946	1947	1948	1949	1950
1. Number of persons who applied for treatment.	4,165	4,638	5,125	5,413	5,473
2. Advice Cases ...	706	602	802	775	539
3. Fully treated ...	3,459	4,036	3,775	4,620	4,934
4. Discontinued treatments ...	Nil.	Nil.	548	18	Nil.
5. Percentage of health returns ...	97.19	95.14	96.15	97.20	Awaited.
6. Cases of Hydrophobia ...	Nil.	Nil.	Nil.	Nil.	1
7. No. of anti rabic centres ...	117	128	119	120	129
8. No. of treatments sent to all centres.	9,255	10,568	10,641	11,044	11,468
9. No. of treatments sent to Vety. Surgeons.	632	648	555	675	606

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

TABLE II:—*Number of anti-rabic treatments issued to different antirabic treatment centres during the years 1949 and 1950, and the bulk supplies made to indentors, not usually dependent on this Institute for supplies of antirabic vaccine.*

Month.	No. of treatments for human use.		No. of treatments for vety. purposes.		Bulk supplies in ml.	
	Year 1949	Year 1950	Year 1949	Year 1950	Year 1949	Year 1950
January	842	971	32	47	1,000	
February	753	1,401	43	38		
March	711	996	43	75	9	
April	1,127	1,084	95	43		
May	825	1,125	66	47	3,330	
June	1,069	913	54	69	9,000	
July	808	1,023	88	32		
August	969	971	67	55		
September	1,147	1,048	72	54		
October	736	778	33	46		220
November	1,134	931	36	37		840
December	933	763	41	63		1,250
Total	11,044	11,468	675	606	14,230	2,310

It would be observed that 11,463 treatments were issued during the year under report for human use and 606 for veterinary purposes. The figures emphasize the popularity and demand for Semple's vaccine from this Institute for both human and veterinary purposes.

The following new anti-rabic treatment centres were opened during the year 1950 bringing the total of such centres dependent for the vaccine on this Institute to 129 :—

- (1) The Associated Cement Co. Ltd., Lakheri Works, Lakheri.
- (2) The Walchandnagar Industries Ltd., Walchandnagar, Poona.
- (3) P. W. Hospital, Jamkhandi.
- (4) Cottage Hospital, Dakor.
- (5) The Associated Cement Cos. Ltd., Kymore.
- (6) G. I. P. Railway Hospital, Bina.
- (7) The Civil Hospital, Upleta.
- (8) The Civil Hospital, Hoshangabad.
- (9) The Civil Hospital, Dhoraji.

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

TABLE III—*Source of brains received for rabies from Bombay City, adjoining suburbs and outstations in 1950.*

		Positive.	Negative.	Unfit.	Total.	Percentage.
Bombay City	...	35	26	2	63	66.81
Suburbs	...	1	1		2	2.11
Outstations	...	10	17	3	30	31.58
Total	...	46	44	5	95	100.00

TABLE IV—*Statement showing the classification of different animals with the result of their brains examination for rabies during 1950.*

	Animals	Positive.	Negative.	Unfit.	Total.
Dog	...	43	43	5	91
Goat	...	3			8
Fox	...		1		1

(d) *Yellow Fever Vaccine and Inoculation.*—The yellow fever diagnostic unit of the Haffkine Institute continued its work of Yellow Fever Inoculations and testing of the potency of Yellow Fever Vaccine stocked on behalf of the Government of India. 16 batches of the Rockefeller Foundation Yellow Fever Vaccine were tested for potency of their viral content during the year, the number of tests performed being 40. Repeated potency tests on several batches have shown that the potency of the vaccine is retained for much longer period than one year. Some batches have, so far, been found to be potent for even three years and more.

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

TABLE V.

Month.	No. of persons immunised.		Month.	No. of persons immunised.			
	1949	1950		1949	1950		
January	...	384	402	July	...	457	607
February	...	802	876	August	...	801	966
March	...	563	686	September	...	592	639
April	...	1,356	856	October	...	427	725
May	...	651	715	November	...	535	681
June	...	618	569	December	...	649	589
Total for 1949...		7,835	Total for 1950...		8,261		

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

Centre	Quantity of vaccine supplied in doses.
1. The Chief Medical Officer, Porbander	460
2. The Port Health Officer, Jamnagar	400
3. The Medical Research Officer, Ceylon	4,000
4. The Director-General of Medical Services, Noa-Goa.	600
5. The Provincial Public Health Laboratory, New Delhi.	480
6. The All-India Institute of Hygiene and Public Health, Calcutta.	3,000
7. The Air Port Health Officer, Santa Cruz	720
Total	9,660

(e) *Research Work*.—1. Random samples of Rabies Vaccine were assayed with Habel's technique. Samples examined showed that the vaccine possessed a high degree of potency.

2. Keeping qualities of Semple's vaccine were studied further.

3. Several mouse potency tests for yellow fever vaccine were conducted. Many factors influencing the results of this test were studied. In particular, the effect of the type of diluent, age and breed of the mice, emperature of the room in which mice were kept, etc., etc. The work is in hand.

4. Common laboratory contaminants were studied to ascertain the variety commonly met with in the laboratory. Acid digest broth, casein hydrolysate and the Fluid Thioglycolate media were used for the purposes of comparison and with a view to finding the medium of choice for use in the sterility test for aerobic contaminant. The work is in progress.

XI. BLOOD BANK DEPARTMENT.

During the year under report the activities of the department were carried on as usual.

The desivac blood plasma drying equipment has been put into successful operation and the product dried on this machine is quite satisfactory. It has been found that presence of an Electro-mechanical Engineer with a skeleton staff is essential not only to run this plant and a number of others in the Institute, but to maintain them at all times in good condition, as there are many complicated mechanisms without automatic safety gadgets that require intelligent supervision all the time the plants are working.

As usual all the blood samples were grouped and the Kahn Tests were performed in this department. The donors were screened for their fitness by determining Hb by CuSO_4 specific gravity method.

The following is the account of the work carried out in the department during the year under report :—

During the year, 2,114 blood samples were tested for Kahn Test of which 348 samples were positive :—

7,250 ml. of high agglutinin titre blood were supplied to the Diagnostic Department for preparation of blood grouping sera.

1,880 blood donors were screened by the CuSO_4 sp. gr. method for fitness to give blood out of which 979 donors were rejected.

The batches of housemen appointed at J. J. Group of Hospitals were trained in the Blood Transfusion Technique.

All Medical Officers appointed in charge of B.T.S. Units of the various hospitals of the province were also trained in the Blood Bank Technique.

The following statements show the work of the department during the year 1950 :—

TABLE No. 1—Showing the number of blood donors.

Centre.	Type of donor.	No. of blood donors.	Total.
Haffkine Institute	Paid	897	...
	Voluntary	36	
	Jail donors	465	
Indian Red Cross Centre	Paid	661	...
	Voluntary	14	
Other Hospitals	Voluntary	93	93
Grand Total ...			2,166

TABLE No. II—*Showing the blood and plasma yields.*

	Product.				Quantity in litres.
Blood	...	...	...	...	559.475
Liquid Plasma	...	...	...	...	292.000
Dried Plasma	...	...	...	...	298.250

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

	Nature				Quantity in litres.	Bottles.
Sold	...	...	...	...	315.250	1,261
Free distribution	...	...	...	...	95.000	380
Total				...	410.250	1,641

The work in connection with medico-legal serology was continued in collaboration with the Chemical Analyser and the technique of detecting and grouping blood stains has been perfected. Highly potent and specific anti-M and anti-N sera have been prepared and this has enabled us to carry out paternity test and these tests are being performed on the cases referred to the Chemical Analyser.

The work of collection of sera suitable for Rh testing is being pursued. All the cases reported by the Public Hospitals of Bombay for Rh sensitization are being investigated. One case of such sensitization has been detected and the serum has been collected from this case to use it as a testing serum, as the antibody titre of the same was quite high.

XII. TRAINING OF HEALTH PERSONNEL.

This Institute plays an important part in dissemination of scientific knowledge by affording facilities to persons desirous of learning different types of techniques either for the prosecution of researches or for routine work in their different spheres of activities. In addition short courses of training of a preliminary nature are given to public health personnel with a view to enable them to carry on their disease control work efficiently. Following is the list of persons for whom training was given in the various departments during the year 1950:—

Serial No.	Name.	Designation and Address.	Department in which trained.	Duration of training.
1	Shri R. Mukundan, B.Sc.	Travancore University Research Scholar. University of Travancore, Trivendrum.	Nutrition	... 29th December 1949 to 12th January 1950.
2	Dr. B. D. Wani, M.B. B.S.	B.M.S. Class II Officer, Sassoon Hospitals, Poona.	Blood-Bank	... 4th April 1950 to 18th April 1950.
3	Dr. M. M. Khilnani, M.B.B.S.	B.M.S. Class II Officer, Civil Hospital, Ahmedabad.	Blood-Bank	... 15th April 1950 to 28th April 1950.
4	Dr. R. M. Nadkarni, M.B.B.S.	Medical Officer, Sayaji General Hospital, Baroda.	Blood-Bank	... 9th May 1950 to 16th May 1950.
5	Dr. L. N. Banarji, M.B.B.S.	Bacteriologist, Bihar Drug Control Laboratory, Patna (Bihar).	Vaccine, Antitoxins and Sera, Pharmacology, Clinical Pathology and Virus Diseases Including Rabies.	8th April 1950 to 7th October 1950.
6	Dr. (Shrimati) Lila P. Shastani, M.B.B.S.	B.M.S. Cl. II Officer, Cama and Albless Hospitals, Bombay.	Blood-Bank	... 26th May 1950 to 9th June 1950.
7	Dr. D. T. Parnaik, G.B.V.C.	Assistant Bacteriologist, Bombay Veterinary College, Bombay.	Vaccine, Clinical Pathology and Antitoxins and Sera.	28th March 1950 to 23rd June 1950.
8	Dr. T. H. Parikh, M.B. B.S., B.M.S. Cl. II.	Blood Transfusion Officer, G. T. Hospital, Bombay.	Blood-Bank	... 2nd December 1950 to 30th December 1950.
9	Dr. T. B. Sonavane, M.B.B.S.	B.M.S. Cl. II Officer, J. J. Group of Hospitals, Bombay.	Blood-Bank	... 12th December 1950 to 30th December 1950.
10	Shri K. S. Vardhan, M.Sc.	Junior Scientific Assistant, Central Drugs Research Institute, Chattr Manzil Palace, Lucknow.	Pharmacology	... Six months from 3rd October 1950.
11	Dr. B. Deodass	Medical Officer, The Associated Cement Co., Ltd. Lakheri.	Virus Diseases including Rabies.	1st February 1950 to 7th February 1950.
12	Dr. P. D. Agarwala	Assistant Surgeon, G. I. P. Rly. Hospital, Bina.	Do.	... 3rd April 1950 to 8th April 1950.
13	Dr. L. D. Shah	Medical Officer, Walchandnagar Industries Ltd., Walchandnagar, district Poona.	Do.	... 8th August 1950 to 16th August 1950.
14	Dr. Ambedi	Medical Officer, Kymore Cement Works, P. O. Kymore (C.P.), via Jukehi, G.I.P. Rly.	Do.	... 21st August 1950 to 30th August 1950.

XIII. CRITICS CLUB.

The activities of the Critics Club continued as before. Weekly meetings were held every Friday in which current Scientific subjects were reviewed by the members of each departments by turns. As a large number of members of the Club were new entrants to the Institute, it was thought advisable to acquaint them with the routine working of all the departments, and with this in view all the heads of the departments in turn explained the routine working of the respective departments.

During the year 37 weekly meetings were held when current scientific literature was discussed. Some of the distinguished scientists who visited the Institute during the year addressed the Club e.g., Professor M. Brumpt, who spoke on vaccine on B. C.G. and Professor Edwards on Fortification of Rice with Vitamin B₁.

Farewell functions were held on the eve of the departure of Major-General S. S. Sokhey to Geneva to take up the appointment of Assistant Director-General of World Health Organisation, and on the retirement of Dr. J. P. Menezes, B.M.S.(II).

Individual talks were also given by the members on the subjects mentioned below :—

- (1) Aureomycin and Chloromycetin in Experimental Plague by Mr. M. K. Habbu.
- (2) Studies on Antirabic Vaccine by Col. J. R. Dogra.
- (3) Chemotherapy of T.B. by Dr. K. Ganapathi.

XIV—INCOME AND EXPENDITURE.

The following table shows the income and expenditure of the Haffkine Institute for the year 1950 :—

Receipt.		Expenditure.	
	Rs.		Rs.
XXVII—Medical—		38—Medical—H.—Bacteriological Laboratories—	
Sale Proceeds of Sera and Vaccines and Other Miscellaneous Receipts ...	21,62,742	H-1. Haffkine Institute.	
		H-2. Pasteur Institute :—	
		Pay of Officers ...	2,05,497
		Pay of Establishment ...	8,76,375
		Allowances, etc. ...	3,29,698
		Contingencies ...	12,55,898
		Research Scholarships ...	1,500
Total Receipts ...	21,62,742	Total Expenditure ...	21,68,968

N. B.—(i) No expenditure was incurred under H-2 Pasteur Institute this year.

(ii) A sum of Rs. 25,098-8-0 which was accepted by the parties was not adjusted by the Accountant General, Bombay, in 1949-50 accounts. The same was adjusted by the Accountant General, Bombay, in June 1950 accounts. Hence the apparent excess of expenditure over receipts as exhibited above.

Part II.

RESEARCH WORK

I. TERRAMYCIN IN THE TREATMENT OF EXPERIMENTAL PLAGUE INFECTION
(WAGLE AND HABBU). •

This crystalline new antibiotic is reported to be active against a variety of gram negative organisms including *S. typhosa*, staphylococcus (*aureus*), streptococcus group and other important group of pathogenic organisms. The drug is also reported to show low toxicity and possesses the great advantage that it can be administered orally.

Experiments were, therefore, put up to assess its value in experimental plague infection in mice.

4 groups of 10 mice (25-30 g.) each were given a test infective dose containing 174 organisms. 72 hours after the induction of infection treatment with Terramycin was started. The drug emulsified in 10 per cent. gum acacia solution so that 0.5 cc. of the suspension contained the requisite dose of the drug was given orally. The first group of 10 mice was given 1 mg., the 2nd group 2.0 mg., the third group 4.0 mg., and the last group 8.0 mg. The drug was given to all the groups every 6 hours for 10 days, except during the first 12 hours when it was given every 3 hours. In addition to these 4 groups of 10 mice a group of 12 mice was kept as controls.

The results given in the attached table show that when the drug was given in inadequate dosage most of the treated animals survived only as long as the drug was being given and they started dying 4 days after the stoppage of the drug. In adequate dosage, however, no deaths took place even after the drug was stopped. From the table, it is clear that a dose of 8.0 mg. given every 6 hours for 10 days is necessary to get the maximum effect of the drug, i.e. a total of 336 mg.

Under similar conditions 42 mg. of Aureomycin and 336 mgs. of Chloromycetin both given orally are required to get the same effect. Terramycin is, therefore, more or less similar in effect to Chloromycetin so far as its treatment in experimental plague in mice is concerned.

*Result of treatment in experimental plague infection in mice
with Terramycin.*

Dose of the drug.	No. of organisms in the test infective dose.	No. of mice used.	Deaths during the period of administration of the drug.	Deaths after the drug was stopped.	Controls.	
					Mice used.	Deaths.
*1.0 mg. four times a day for 10 days.	174	10	1	4	12	12
*2.0 mg. four times a day for 10 days.	174	10	1	2	12	12
*4.0 mg. four times a day for 10 days.	174	10	1	2	12	12
*8.0 mg. four times a day for 10 days.	174	10	1	0	12	12

* During the first twelve hours on the first day the doses were given every 3 hours.

II. CURATIVE VALUE OF AUREOMYCIN BY THE PARENTERAL ROUTE.

(Wagle and Habbu).

It was expected that Aureomycin given by the parenteral route would be quite effective in doses as low as $1/5$ th the oral dose. So experiments were put up to try the effect of Aureomycin by the parenteral route. In order to try the value of this drug on a comparative basis with Streptomycin, the drug was given in this case also subcutaneously every 6 hours, 72 hours after the induction of infection. When a dosage of 0.2 mg. was employed, 9 out of 10 treated mice died within 6 days after the drug administration was started. So the drug administration was stopped after 7 days. The remaining animals also died 2 days after stopping the drug, bringing the mortality to 100 per cent.

When a dosage of 0.5 mg. every 6 hours was employed 3 out of the 5 treated animals died within 5 days after the drug administration was started. The two surviving mice were given the drug for a total period of 10 days. One of the two surviving mice died on the 26th day with acute plague infection, i.e., 13 days after the drug administration was stopped, bringing the total mortality to 80 per cent.

In both the experiments deaths among controls were 100 per cent. From these experiments it is clear that the drug is not effective when given by the subcutaneous route.

III. CASEIN HYDROLYSATE T.A.B. VACCINE.

(Wagle and Kulkarni).

Encouraged by success in producing highly potent plague and cholera vaccines in the casein hydrolysate medium, we explored the possibility of preparing the T.A.B. Vaccine also in a liquid medium. As typhoid bacilli are reported to require a number of essential and accessory growth factors and which are lacking in the casein hydrolysate which we use for the production of plague and cholera vaccines, we tried the casein hydrolysate medium reported by Gladstone and Fildes (1940) with certain modifications.

Method.

The medium used consisted of a mixture of acid digest, and pancreatic digest of casein in equal parts, buffered with sodium glycerophosphate and fortified with sodium lactate. The pH of the medium was adjusted to 7.4.

500 cc. of this medium were taken in a litre flask and seeded with the organism. The culture was incubated at 37°C for one week with continuous aeration. The culture was killed and detoxicated with 0.1 per cent. formalin and preserved by adding 1.5 mgs. per cent. of phenyl mercuric nitrate. The three organisms *Bact.typhosum*, *Bact.paratyphosum* A and *Bact.paratyphosum* B. were grown separately and then mixed in proper proportions.

Potency Test of Vaccine.

Active Immunity Test.—Groups of mice were immunised with graded quantities of this vaccine, given in two doses at an interval of one week. A week after the 2nd vaccine dose, a challenge dose of about 10,000

organisms (10 m.l.d.) of *Bact.typhosum* Ty2 suspended in 5 per cent. mucin was given intraperitoneally. The animals were observed for 3 days and the 50 per cent. mouse protection dose evaluated. Along with these tests, agar grown vaccines from the same strains were also put up for comparison. The results of a number of tests which closely agree indicated that the casein hydrolysate T.A.B. Vaccine was as good as the agar grown vaccine.

Passive Immunity Tests.—Rabbits were immunised by injecting the same doses of casein hydrolysate and agar grown T.A.B. Vaccines and the immune sera titrated for their protective power.

Groups of mice were given graded doses of the sera and were challenged immediately with 100,000 organisms of *Bact.typhosum* Ty2 (100 m.l.d.), suspended in 5 per cent. mucin. As in the above experiments the mice were observed for 3 days and mouse protective doses evaluated. Two independent tests were carried out and the results showed close agreement. The average mouse protective dose of the serum prepared against agar vaccine was 3.6 times the mouse protection dose obtained with the serum prepared against casein hydrolysate T.A.B. Vaccine. It is thus evident that casein hydrolysate T.A.B. Vaccine is as good as agar grown vaccine when tested by the active immunity test, but is found to be much superior when tested by the passive immunity test.

Toxicity Tests.

After ascertaining that the casein hydrolysate T.A.B. Vaccine possessed high mouse protective value, the next important thing investigated was the toxicity of the vaccine. We tested the toxicity of the vaccine by injecting subcutaneously in 4 mice (wt. 26-27 g.), 0.5 cc., 0.75 cc., 1.0 cc., and 1.5 cc. of the vaccine in a single dose; for comparison agar grown T.A.B. vaccine also was tested in a similar way. The mice were observed for 3 days.

None of the mouse in any of the groups died nor showed any serious toxic symptoms. It is thus evident that both the casein hydrolysate T.A.B. Vaccine and the agar grown T.A.B. Vaccine have very low toxicity. Further work is continuing.

IV. EVALUATION OF IMMUNOGENICITY OF HAFKINE INSTITUTE DIPHTHERIA TOXOID WITH REFERENCE TO THE PROPOSED INTERNATIONAL STANDARD DIPHTHERIA TOXOID.

(Lahiri).

Several batches of crude diphtheria toxoid, which were prepared for human immunization, were tested in guineapigs with reference to the freeze-dried international standard. The immunizing doses were fixed in terms of their Lf. The immunised guineapigs were challenged with 20 lethal doses of the test diphtheria toxin, 28 days after immunization. Compared Lf. per Lf, all the batches of the crude toxoid tested showed over twice as potent as the highly purified reference standard diphtheria toxoid. Though the Lf of the different batches of the crude toxoid differed from one another, given in the same Lf dose to the guineapigs, all the batches immunised equally well. Thus, it was indicated that

with the same medium, strain, and technique of production, toxoids of the same quality were produced, though each batch differed in the quantity of the specific toxoid measured by their Lf value.

V. NATURAL NON-SPECIFIC SYNERGISTS AND ANTAGONISTS IN CRUDE DIPHTHERIA TOXOID.

(Lahiri).

It was shown that though the different batches of diphtheria toxoid produced in the same medium, showed the same immunogenicity per Lf, the crude toxoid, produced in different media, differed in their immunogenicity per Lf. This was experimentally shown to be due to the presence of natural non-specific synergists and antagonists present in the crude toxoid along with the specific toxoid. Synergists enhanced the immunogenicity of the specific toxoid, thereby showing advanced overall immunogenicity; while antagonists reduced the specific immunogenicity, thereby showing a very poor overall immunogenicity.

Presence of these non-specific synergists and antagonists was shown in two different ways: (1) The specific toxoid from several crude toxoids, prepared in different media, was removed by flocculation with antitoxin. To these detoxoided supernates was then added constant quantities of a highly purified freeze-dried diphtheria toxoid of foreign origin. Determination of immunogenicity of these hybrid samples indicated the presence of synergists and antagonists in the detoxoided supernates; (2) The specific toxoid was purified from crude toxoids by precipitation with ammonium sulphate. The impurities were also saved, and a portion of the purified toxoid was reconstituted to the original impure form with the addition of these separated impurities. Immunogenicity of (a) the crude toxoid, (b) the purified toxoid, and (c) the reconstituted purified toxoid, was compared. The results showed that (a) the crude toxoid contained synergic impurities, (b) removal of which during purification reduced the immunogenicity of the purified toxoid, (c) but addition of these synergic impurities to the purified toxoid while reconstituting it, made good the loss in immunogenicity suffered by the purified toxoid due to removal of these impurities.

VI. CONCENTRATION OF ANTITOXINS BY PEPSIN DIGESTION.

(Rao).

Antitoxins purified after a preliminary digestion by pepsin are known to lose their property to act antigens though they still retain the power to combine with and neutralize the toxins both *in vitro* and *in vivo*. Pepsin digested antitoxins thus lose to a large extent their ability to produce in human beings hypersensitization and serum allergy.

The pepsin digestion method of Parfentjev was modified and to some extent simplified. Briefly, the process developed consists in the digestion of horse plasma with pepsin at pH 4.2 for 24 hours at 37°C; under these conditions most of the albumin of the plasma is digested while the globulins including antitoxic globulin are slightly degraded. The degraded antitoxic globulin still retain its power to combine with its toxin. Ammonium Sulphate to 28 per cent. saturation is then added to the digested plasma and pH is adjusted to 4.4 and the solution filtered. The filtrate which contains the antitoxins is neutralized and Ammonium

Sulphate saturation increased to 50 per cent. The precipitated antitoxins are filtered out, pressed and dialysed.

Antitoxins of Diphtheria, tetanus and antivenenes can be processed this way. The keeping quality of the purified antitoxins are under test. Tests carried out so far show that digested antitoxins are more stable than undigested antitoxins processed in the usual way. The only drawback of the process is that yields are 10 to 15 per cent. lower but the product is about 30 per cent. to 50 per cent. purer. Experiments to increase the yield are in progress.

VII. STUDY OF THE ACTION OF CRYSTALLINE PROTEOLYTIC ENZYMES ON TOXINS, TOXOIDS, ANTITOXINS, VENOMS, ETC.

(Shanta S. Rao).

This study was undertaken with a view to ascertain the chemical constitution of proteins such as toxins, toxoids, antitoxins etc. as well as to devise methods of purification of these substances. The use of pure substances in immunization avoids to a very great extent the allergic reactions that often follow when impure proteins are injected. The enzyme method of purification has a further advantage that though the proteins on which it acts may get partially degraded, they still retain their power to combine with antibody.

The first step in this study was the preparation of crystalline proteolytic enzymes. The crystalline state is one of the best criteria for purity and reliability. Crystalline trypsin and crystalline chymotrypsin were prepared according to the method of Northrop with slight modifications, from bovine pancreas. Crystalline egg albumin was also prepared. Great difficulty was experienced in its preparation and the first few experiments did not yield any crystalline egg albumin. Only when very fresh eggs were used and certain conditions were strictly adhered to, it was finally possible to prepare it in some quantity. The action of the crystalline enzymes was tried on toxins and toxoids of diphtheria and tetanus. It was found that while crystalline trypsin destroyed toxins of diphtheria and tetanus very readily, it did not act on their toxoids. Since toxoids were found to be resistant to tryptic action, they could be easily purified after digestion of the crude toxoids. Formalinized egg albumin was also found to be resistant to tryptic action. Further work on the purification is being continued.

VIII. TYPHOID FEVER IN THE CITY OF BOMBAY.

(Hazra and Modi).

Free diagnostic aid in cases of typhoid fever continued in operation during the year 1950, as before. The incidence of typhoid fever in Bombay rose again during the months of June, July, August, September and October and the average monthly figures of the calls were as follows:—

Year.	Total.	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov	Dec.
1950	856	48	45	37	49	59	83	81	109	106	114	67	56

As a routine, blood was collected for Widal Test, clot culture, blood culture, Weil-felix Test and agglutination test for brucellosis. Total and differential counts were done only when practitioners asked for the same. The number of samples examined definitely increased owing to greater publicity of the free facilities available at the Institute. No case of paratyphoid B or paratyphoid C infection was diagnosed either serologically or bacteriologically. The ratio of *B. typhosus* to *B. paratyphosus* A strains isolated continued to be 4:1 as compared with that of previous year. The following table illustrates the results for the year 1950:—

Year.	Total samples.	Hospital samples.	Private samples.	Widal - Culture +	Widal + Culture -	Widal + Culture +	Total Cultures + done.	B. typhosus isolated.	B. para A isolated.
1950	2,354	1,542	812	167	524	229	2,747	339	88

Total number of calls registered was 856, of which 44 were such where in patients' address was incomplete or the patient refused to give blood. Thus 812 blood samples were collected and examined, of which 306 were diagnosed as cases of enteric, of which 148 were bacteriologically positive. Out of 148 cultures, 37 were *B. paratyphosus* A. As a routine, both blood culture (Hartley's Broth) and clot culture (Glycerine-Peptone-Bile) were done on the samples of blood collected each sample being equally divided except in 14 cases where only clot cultures were done because the quantity of blood collected was not sufficient for both purposes. Hence comparative positive results obtained by clot and blood cultures were worked out in 134 cases only. Out of 134 bacteriologically proved cases, both blood and clot cultures were positive in 89, only blood in 20 and only clot in 25.

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

Distribution of calls and enteric cases under typhoid scheme.—There were 711 calls from the city and 101 from the suburbs and this distribution according to wards was as follows:—

	Wards.							Suburbs.	Total:
	A.	B.	C.	D.	E.	F.	G.		
Calls received ...	23	15	77	141	53	221	181	101	812
Enteric cases ...	7	7	31	62	18	87	60	34	306

As judged by the distribution of cases reported by private medical practitioners, it is found that D, F and G showed the highest incidence of enteric cases in the city for the year under report.

IX. ENDOMETRIAL BIOPSY FOR DIAGNOSIS.

(Dogra).

A high incidence of tuberculosis infection of the endometrium where none was suspected, was noticed. A study of the material available in the department was made. Material received at the Institute during 1944-49 was studied. It represented an average cross section of pathological conditions throughout the lower and lower middle class of the population.

Of the 1,217 specimens examined 99 were unfit for report. The remainder 1,118 fell into five more groups shown in the Table I. Groups (1), (2), and (3) need no explanation. Group (4) "other pathological lesions" consisted of cases of abortion, metropathias, senile endometritis etc. etc. Group (5) consisted of cases with some menstrual disorder but in which no pathological lesion was found to account for the complaint.

TABLE I.—*Showing classification of 1,118 endometrial biopsy samples received during 1944-49 at the Haffkine Institute, Bombay.*

Year.	Tuberculous endometritis.	Malignant disease.	Chronic endome- tritis.	Other pathological lesions.	No pathological lesions.	Total of column (1), (2), (3) and (4).	Grand Total.
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1944	...	8	Nil.	20	52	30	110
1945	...	12	1	42	58	36	149
1946	...	7	5	103	57	9	131
1947	...	8	2	88	54	60	212
1948	...	6	2	101	64	48	221
1949	...	10	3	141	74	17	245
Total	...	51	13	495	359	200	1,118

The incidence of tuberculous infection (5.55 per cent.) is considered high. It was observed even where general signs of tuberculous infection were not easily detectable.

Chronic Endometritis is considered a rare condition, but in our series it showed a very high proportion (53.92 per cent.). The pathological lesions frequently observed consisted of relative preponderance of plasma and round cells along with infiltration with polymorphonuclear leucocytes. In very chronic cases fibrous tissue reaction and even distortion of endometrial glands was present. Cases of hyperplasia of endometrium due to hormone therapy or disordered ovarian function and cases of incomplete abortion in which a similar picture may be seen along with a decidual cell reaction and presence of chorionic villi, were carefully excluded. The rather pseudo-inflammatory infiltration that occurs just before menstrual desquamation was also kept in mind. Attention is drawn to this observation to elicit findings of other workers in this field.

X. A STUDY OF THE EFFECT OF 1 PHENYL-1-(2 PYRIDYL)-3-DIMETHYL-AMINO-PROPANE MALEATE (PROPHENPYRIDIAMINE MALEATE) ON THE HISTAMINE INDUCED GASTRIC SECRETION IN CATS.

(Dutta and Tamhane).

It was previously reported by Dutta (1949) that antihistamine Benadryl (Parke Davis & Co., Ltd.) significantly increased the rate of hydrochloric acid secretion from the stomach of atropinised cats during intravenous perfusion of histamine. The present study was undertaken to find out whether the recently introduced anti-histamine compound Propenpyridiamine maleate (Schering Corporation Ltd.) has any effect on the gastric acidity or not. The method described by Howat and Schofield (1948) was followed. Histamine acid phosphate (0.0135 mg/kg/min.) in 0.9 per cent. sodium chloride solution was infused intravenously in atropinised (1 mg/kg) cats for 45 minutes. The total acid secreted during this period constituted the first response. After the secretion had returned to the basal level, histamine acid phosphate was infused for the second time and the total amount of acid secreted constituted the second response. In 10 control experiments in which no anti-histamine was injected, the amount of hydrochloric acid secreted during the first and the second response were 16.19 ml. (N/20) and 19.53 ml. (N/20). The mean difference between the two responses was 3.44 ml. which was not statistically significant. In six experiments 5 mg/kg of Propenpyridiamine maleate was injected intravenously in between the two responses with histamine. The amount of acid secreted before and after the administration of the anti-histamine were 13.4 ml. (N/20) and 13.93 ml. (N/20) respectively. The mean difference between the two responses was not significant statistically. Propenpyridiamine did not alter the rate of hydrochloric acid secretion from the stomach of cats induced by histamine.

XI. BIOLOGICAL ASSAY OF U.S.P. REFERENCE STANDARD OUBAIN ON CATS.

(Deshpande and Dutta).

U.S.P. reference standard Oubain was dissolved in sufficient 80 per cent. alcohol to make 1 in 1,000 and kept at 0°C. All the assays were completed within one month of the preparation of the solution.

Healthy cats of either sex were selected and amongst the females pregnant and lactating animals were not used. Food was withheld for a period of 16 to 18 hours prior to the experiments. The animals were anaesthetised with chloralose (8 cc. per kg. of body weight of 1 per cent. solution given intravenously) preceded by a dose of ether. The blood pressure was recorded through the common carotid artery. The standard solution of Oubain after dilution 1:1.2 with normal saline was injected through the femoral vein at intervals of 5 minutes till the heart ceased beating. The average Minimum Lethal Dose of the Solution of Oubain on 13 cats was found to be 14.88 ± 0.456 , standard error was 0.0288.

XII. BIOLOGICAL ASSAY OF THE SECOND AND THIRD INTERNATIONAL STANDARD DIGITALIS AND THE U.S.P. REFERENCE STANDARD ON GUINEAPIGS.

(Dutta and Deshpande).

One gramme from each of the standard powders was added to 10 ml. of 80 per cent. (by volume) alcohol (sp. gr. 0.864, 15°C/15°C). The mixture was shaken continuously for 24 hours at 26°—30°C by an electric shaker. It was centrifuged and the Tincture Digitalis stored at 0°C. All the assays were completed within one month of the preparation of the tincture.

Healthy male guineapigs (except albinos) bred in this Institute weighing 475 to 575 grammes were selected. The animals had no access to food for 18 to 24 hours before the experiment started. Urethane (25 per cent. in 0.9 per cent. NaCl solution) in a dose of 7 cc. per kg. was injected intraperitoneally. The tinctures were diluted 1 in 12.5 with 0.9 per cent. NaCl and infused slowly into the vein till the heart stopped. The average M. L. D. of the diluted tinctures on 13 animals in each case for the Second and the Third International Standards were 12.91 ml. (standard deviation ± 0.46 , standard error 0.0329) and 12.906 ml. (standard deviation ± 0.54 , standard error 0.3883) respectively. The average M. L. D. for the diluted U. S. P. Reference Standards on thirteen guineapigs was 16.1 ml. (standard deviation ± 0.77 , standard error 0.0535).

XIII. BIOLOGICAL ASSAY OF THE THIRD INTERNATIONAL STANDARD DIGITALIS ON CATS

(Dutta and Deshpande).

One gramme of the Third International Powder was added to 10 ml. of 80 per cent. (by volume) alcohol (Sp. Gravity 0.864, 15°C/15°C). The mixture was shaken continuously for 24 hours at 26°—30°C by an electric shaker. It was centrifuged and the Tincture Digitalis stored at 0°C. All the assays were completed within one month of the preparation of the tincture.

The animals of either sex were selected. They were non-pregnant and non-lactating and apparently free from diseases. These were starved for a period of 16—18 hours before being used. The cats were anaesthetised with chloralose (8 cc. per kg. of body weight of a 1 per cent. solution, given intravenously) preceded by a dose of ether. The tincture made from the Third International Standard Digitalis was diluted 1 in 30 with 0.9 per cent. NaCl and was injected intravenously at intervals of 5 minutes till the heart ceased beating. The average M. L. D. of the diluted tincture on 15 cats was found to be 16.14 ml. (standard deviation ± 1.1 , standard error 0.0963).

XIV. CHEMOTHERAPY OF MALARIA: SYNTHESIS OF QUINOLINE DERIVATIVES.

(Ganapathi and Shah).

In view of the established anti-malarial activities of some of the substitute 4-aminoquinoline compounds such as chloroquin SN 7618, an attempt has been made to prepare a number of quinoline derivatives,

with a view to obtain more active and less toxic antimalarial drugs. Incidentally the chemistry of quinoline derivatives has been studied with regard to the scope of application of the Skraup synthesis and a number of 2-chloro- and -4-chloroquinoline derivatives have been synthesised. The chemical reactivities of the chloro and the amino groups situated at the position 4 of the quinoline nucleus has been investigated.

The amino group in the position 4 of the quinoline nucleus has been found to be rather chemically inert with regard to its interaction with various thiocyanates and cyanogen compounds, although a few acid salts and acetyl derivatives thereof could be prepared.

4-(p-guanidinophenyl)-amino-7-chloroquinoline and 4-(p-N¹-isopropyl-guanidinophenyl)-amino-7-chloroquinoline have been synthesised. In like manner a number of quinolyl-thiazolylamines and substituted quinolyl-pyrimidylamines and 4-phenoxyquinolines have been prepared. A number of substituted 4-chlorolepidines and 4-chloroquinaldines through the appropriate acetoacet-arylamines and substituted crotonates respectively have been synthesised and optimum experimental conditions for the synthesis of 8-nitroquinoline and 6-chloro-8-nitro quinoline in high yields by Skraup synthesis have been reported.

About 125 quinoline compounds and intermediates for the synthesis thereof have been synthesised during the course of the work.

XV. CHEMOTHERAPY OF MALARIA : SYNTHESIS OF P-PHENYLENEBIGUANIDINES.

(Ganapathi and Kulkarni.)

In view of the encouraging results obtained with biguanidines, particularly with N¹-p-chlorophenyl-N⁶-isopropyl-biguanidine hydrochloride (Paludrine) in the treatment of Malaria, it was felt desirable to investigate the antimalarial activity of other biguanidines wherein substituted guanidine groups are attached to a phenyl nucleus in the 1:4 positions. With this end in view, p-phenylenedithioureas were prepared by the customary methods of condensing requisite isothiocyanates with the aromatic amino groups of p-phenylenediamine. These dithioureas failed to yield the desired biguanidines by interaction with mercuric oxide and alcoholic ammonia, however the s-methyl-derivatives of the said dithioureas prepared by the action of methyl-iodine could be successfully converted into the required biguanidines by interaction with alcoholic ammonia.

During the course of the work 13 substituted isothiocyanates were prepared by the interaction of the requisite amine in carbon disulphide a method though generally known had been but little investigated so far. During the course of this work 20 new substituted p-phenylenedithioureas, their corresponding s-methyl derivatives and finally the corresponding 20 new substituted p-phenylenebiguanidines have been prepared. Among several compounds prepared during the course of this work 85 new compounds have been synthesised. The structure of the guanidino derivatives and some of the intermediates prepared in connection therewith have been established by synthesising a few of the representative members by different routes and comparing the products so obtained.

XVI. CHEMOTHERAPY OF BACTERIAL INFECTIONS : SYNTHESIS OF PTERIDINES.

(Ganpathi and Palande).

In virtue of the establishment of the fact that Folic Acid is an essential metabolite for several organisms it was felt desirable to investigate whether or not pteridines substituted with various aryl and aliphatic groups would act as antibacterial agents. With this end in view about 15 substituted pteridines have been synthesised. N'-arylthioureas were condensed with cyan acetic ester in the presence of sodium butoxide to obtain 3 aryl-4-amino-thiouracils. The so prepared thiouracils on treatment with nitrous acid followed by reduction afforded the corresponding 4-5-diamino 3-aryl-thiouracils which in turn were condensed with various alpha-diketones to obtain the requisite thio-pteridines.

In like manner starting from N'-aryl ureas, the corresponding pteridines were obtained as outlined above.

During the course of this work 52 different compounds have been synthesised apart from several other intermediates that had to be prepared incidentally.

XVII. CHEMOTHERAPY OF TUBERCULOSIS : SYNTHESIS OF PARA-AMINO SALICYLIC ACID AND DERIVATIVES THEREOF.

(Shirsat, Sabnis and Ganpathi).

Five different methods for the synthesis of para-amino benzoic acid have been studied with regard to their suitability for the commercial manufacture of para-amino salicylic acid, (PAS). Of the various methods investigated, that involving the use of phenyl acetic acid as the starting material appears to be the most practicable and economical. Phenyl acetic acid is nitrated to 2:4-dinitrophenyl acetic acid which is then esterified with an aliphatic alcohol by customary methods. The ester is then converted into 6-nitro-benz-isoxazole-3-carboxylic acid alkyl ester which on boiling with alkali affords 2-hydroxy-4-nitro-benzonitrile. The nitrile on hydrolysis by boiling with about 60 per cent. sulphuric acid affords 2-hydroxy-4-nitrobenzoic acid which on reduction by customary methods affords 2-hydroxy-4-amino benzoic acid. The procedures reported in literature have been further improved upon during the course of this work as a result of which a large scale manufacture of this important drug by the adoption of the improved process appears to be feasible. Further work is in progress.

A number of derivatives of para amino salicylic acid have been prepared with a view to obtain more effective drugs for the treatment of tuberculosis. Thus 2-alkoxy-PAS, 2-phenoxy-PAS, 2-benzyloxy-PAS and their corresponding alkyl esters have been prepared. Alkyl esters of PAS have been prepared with a view to ascertain whether the lipid solubility of such compounds would render them in any way less toxic and more effective than PAS. A number of alkyl amides of PAS have also been prepared since in many cases the amides of acids often exhibit the same physiological actions as exhibited by the parent acids. It is also hoped that the greater stability of the PAS-amides and the lipid solubility imparted to the molecule in virtue of the alkyl groups attached to the

nitrogen of the amido group may prove to be an advantage over the parent molecule of PAS. N-sulphanilyl-PAS and various alkyl esters and amides thereof have also been synthesised. Further work in connection with the preparation of acyloxy-PAS, acyl-amino-PAS and the corresponding di-acyl derivatives of PAS is in progress.

XVIII. CHEMOTHERAPY OF INTESTINAL INFECTIONS :

(Shirsat, Sardesai and Ganpathi).

In virtue of the reported activity of N -(4-substituted) phenyl-sulphanilamides as antibacterial agents a number of similar compounds having the residual hydrogen attached to the nitrogen at N¹ position in such compounds replaced by alkyl groups, particularly by the iso-propyl group have been synthesised. These compounds as should be expected are insoluble in aqueous alkali and indeed have very feeble basicity inasmuch as these compounds do not form stable salts with mineral acids. In virtue of the insolubility of these compounds in alkali, it is hoped that some of these compounds may prove to be useful for the treatment of intestinal infections since very little, if any, of these compounds are expected to be absorbed in the system via the alimentary canal. Further work with regard to in vitro and in vivo tests with these compounds is in progress.

XIX. STUDIES IN CHEMISTRY OF THIAZOLES.

(Shirsat and Ganpathi).

When p-acetamino-benzene-sulpho-chloride is condensed with 2-amino thiazole in aqueous medium using sodium hydroxide, sodium carbonate, sodium bicarbonates, calcium carbonate etc., for fixing the hydrochloric acid liberated during the course of the reaction, one gets 2-diacetsulphanilyl-amidothiazole. In this compound two acetsulphanilyl groups are attached to the nitrogen in position 2 of 2-aminothiazole. To have the maximum yield from the reaction involved it is necessary to employ 2 moles of p-acetaminobenzene-sulphonyl chloride for one mole of 2-aminothiazole. This condensation product on boiling with acids or alkalies generates one mole of sulphathiazole and one mole of sulphanilic acid. It would be obvious that out of the two moles of p-acetaminobenzene sulphonyl chloride used for the formation of the abovementioned dicondensation product only one mole is really effective in giving sulphathiazole, the other mole being wasted in its being ultimately converted into sulphanilic acid. This process for the preparation of sulphathiazole has been primarily covered in the Indian Patent specification Number 29093, dated 1st October 1942, held in the name of the Director, Haffkine Institute. As a result of further research work carried out in this direction, it has now been found out that it is possible to fully utilise all the amount of p-acetaminobenzene-sulphonyl chloride for the formation of sulphathiazole proper without any part thereof being rendered useless in the form of sulphanilic acid.

When the above stated dicondensation product is suspended in a solution of 2-amino-thiazole in water, alcohol, acetone or a mixture thereof and the mixture thereupon heated for some time, one of the acetsulphanilyl

group in the said discondensation product leaves the parent molecule and combines with the freshly added 2-aminothiazole to form one fresh mole of acetsulphathiazole. Thus starting from 2 moles of p-acetaminobenzenesulphonylchloride one gets two moles of sulphathiazole. This improvement in the process has been extensively studied with regard to various experimental conditions such as temperature, heating, solvent used, proportion of 2-aminothiazole employed etc., with the result that a yield of sulphathiazole nearly double that hitherto obtained by the older process has been achieved. The process has now been covered under Indian Patent No. 39684, dated 28th April 1948, held in the name of the Director, Haffkine Institute.

XX. INTERRELATION OF NIACIN AND TRYPTOPHANE.

(Aroskar).

The disease pellagra has been found to be rather common among the maize-eating people of the world and it can be prevented or cured by nicotinic acid therapy. During the numerous investigations that have been carried out with a view to elucidating this mechanism, it is reported that in albino rats the growth deficiency caused by maize feeding can be corrected by supplements of either nicotinic acid or tryptophane. From these and other investigations it is believed that tryptophane in some way is converted into nicotinic acid in the body of the rat. But some workers from U. S. A. and France have reported that maize does not produce any growth deficiency when fed to rats and supplements of either nicotinic acid or tryptophane do not result in greater growth rates. But there is general agreement in the observation that tryptophane feeding does replace the need for nicotinic acid though the findings about the mechanism of action are controversial.

With a view to review this problem critically, experiments have been conducted, feeding yellow maize to growing albino rats. The results obtained clearly show that the variety of the yellow maize used does not produce any deficiency in the rats as observed by the growth rates which favourably compare with casein-fed rats and also by the failure to obtain increase in the growth-rate by supplementing niacin or tryptophane in the diet. The results of analyses of the tissues show that tryptophane supplement causes an increase in the niacin content of liver of rats on casein as well as maize diet, though the supplement does not cause any increase in the growth-rate of rats. Study of the effects of supplements further, show that tryptophane causes this increase in the level of niacin, only in the liver and not, in other tissues like muscle, and that the action is possible only when the supplement is above a certain limit (*viz.*, 100 mg. of DL- or 50 ml. of L-tryptophane). It is also observed that the highest concentration of niacin in the liver is reached almost immediately after tryptophane feeding, the level reaching a certain limit, later feeding only maintaining the level to that constant value.

It has been stated by various workers that maize feeding causes an increase in the demand for niacin and that tryptophane accelerates the synthesis of niacin in the rat. Experiments have been carried out to study the excretion of niacin under the action of tryptophane feeding. The results obtained show that tryptophane causes an increase in urinary

niacin value. This can be due to two reasons : that the tryptophane is converted to niacin in the liver and after raising the level to a constant value excess niacin formed is excreted or that the conversion takes place in the intestine from where the niacin is absorbed by the body and excess excreted in the urine. In order to investigate this, sulfaguanidine has been added to the diet of tryptophane-fed rats to suppress the micro-organisms of the intestine, and this is found to cause an increase in niacin excretion showing thereby that the conversion does not take place in the intestine but probably in the liver as otherwise there would have been a reduction in urinary niacin after suppressing of the intestinal flora by the sulfa drugs.

Since in our investigations it is found that maize does not produce any growth-deficiency, in albino rats, it was thought desirable to study whether feeding of some Indian pulse proteins of low tryptophane and nicotinic content would produce any growth deficiency in rats. The results of growth rate show that except Bengal gram, other pulses like black gram, green gram, red gram and lentil cause a much lower growth-rate than casein and that tryptophane feeding does not cause any enhancement in the growth rate. This shows that low tryptophane is not always the cause of the deficiency in growth. The exact reason of low growth with the pulses is, however, not understood.

XXI. EXPERIMENTAL HEPATIC CIRRHOSIS.

(Radhakrishna Rao, Dutta & Krishnan).

The influence of Tocopherol on the production of massive hepatic necrosis in rats.—In 1944 Himsworth and Glynn claimed to have produced massive hepatic necrosis in rats by low protein diets and the factor in protein, lack of which caused necrosis was, subsequently (1945) shown by them to be cystine. Since then it became clear that, even when similar diets are used there are discrepancies between the results obtained in the different laboratories. Radhakrishna Rao (Nature, 161, 1948) enumerated the different factors which might be responsible for these discrepancies. In the meantime, experiments of Schwartz (1944) and Gyorgy (1947) seem to have indicated the importance of tocopherol content of the dietary fat on the incidence of liver necrosis in animals. In order to throw further light on this point, the following experiments were planned and conducted.

(a) *Prevention of necrosis of the liver of rats by tocopherol.*—24 rats were distributed evenly, as far as possible with respect to age, weight, sex and litter mates, into two groups so that each group contained 12 rats. The two groups of rats with an initial body weight of about 70 g. were placed on the 'yeast protein diet' similar in composition to that which Himsworth found to be most effective in producing hepatic necrosis. Each rat was given daily 8 g. of the diet. One group received a supplement of alpha-tocopherol (1 mg. per rat per day) and to the second group, which served as control, no tocopherol was given. The body weights of the animals were recorded weekly. A record of the day to day food consumption was also maintained.

The animals were fed on the experimental diet for a period of 275 days, after which the rats from both the groups were killed at regular intervals. The livers of the animals were examined histologically and chemical analysis of the moisture, protein, fat and ash content were also carried out.

As glutathione plays an important role in the oxidation-reduction metabolism of the liver, it was felt that the estimation of glutathione may throw some light on the chemical composition of the liver under different dietary conditions. The glutathione content of the liver was estimated by a modified method of Woodward and Fry (1935).

The results of the analysis are under compilation. The following table will, however, give a preliminary idea :—

Groups.	Initial body wt. g.	Final body wt. g.	Liver wt. g.	Liver water per cent.	Liver fat per cent.	Liver produced glutathione mg. per 100 g. liver.
Vitamin E group ...	73.1	161.6	5.906	70.02	2.76	100.4
Control group ...	73.1	151.5	5.544	70.52	3.15	101.0
Normal rats on 'Stock Diet'.	...	155.2	5.646	69.47	2.42	221.3

The animals of both the groups did not show any marked differences in appearance or behaviour during the experimental period.

Macroscopically, the livers of both the groups of animals did not show any significant variation from those of normal rats.

Histological Findings.—Preliminary histological examination has shown that in the livers of rats receiving tocopherol as supplement, the parenchyma is slightly vacuolated in appearance. In a few livers, fine bands of connective tissue were seen spreading out from the central veins. In the control group of animals, which did not receive any tocopherol as supplement, the parenchymal cells of the liver showed only the slightly vacuolated appearance. No other pathological changes were seen.

In both groups of rats no necrosis of the liver cells was found. This is in contrast to the findings of Himsworth and others.

(b) *The effect of Pre-experimental diet on the development of massive hepatic necrosis in rats.*—Weaning rats with an initial weight of about 30 g. from 5 litters were distributed evenly with respect to sex, weight and litter mates into two groups, so that each group contained 18 rats. Both the groups were put on a diet consisting of alcohol extracted casein, yeast and lard (as dietary fat) in order that the diet is deficient in tocopherol. One group was given 1 mg. of tocopherol, per rat per day, while the other served as control. After 120 days on these diets, both the groups were placed on the yeast protein diet of Himsworth without any supplement of tocopherol. A weekly record of the body weight of the animals, as well as a record of the daily food consumption was maintained.

After 150 days on the necrogenic "yeast protein diet" the animals from both the groups were killed at regular intervals. Histological and chemical examination of the livers were carried out as described in the

previous experiment. The results are under compilation. The following table, however, will give a preliminary idea :—

Groups.	Initial body wt. g.	Final body wt. g.	Liver wt. g.	Liver water per cent.	Liver fat per cent.	Liver reduced glutathione mg. per 100 g. of liver.
Vitamin E group ...	38.5	135.6	4.827	69.8	3.84	102.2
Control group ...	38.9	126.8	4.118	71.3	3.12	95.1
Normal rat on stock diet .	...	155.2	5.646	69.5	2.42	221.3

During the experimental period, the animals did not show any abnormalities.

Microscopically, the livers of both the groups of animals were similar to normal except for a little palor seen in a few animals.

Histological Finding.—Preliminary histological examination has shown that the liver parenchyma of rats receiving tocopherol as supplement, showed a normal histological pattern except for a slight vacuolation seen in a few animals. The histological picture was also similar in the control group. No other pathological changes were seen.

In both the groups of rats, necrosis of the liver cells, as described by Himsworth, was not seen.

On the basis of the above experiments, it can now be said that with the "yeast protein diet" of Himsworth, it is not possible to produce necrosis of the liver. While this report was being prepared, we have come across a recent paper by Paul Gyorgy (1950) in which it is stated that necrosis of the liver can only be produced by certain types of yeast.

XXII. STUDIES ON BLOOD PROTEINS IN HEALTH AND DISEASE, AND THE EVALUATION OF DIFFERENT METHODS OF ESTIMATION OF BLOOD-PROTEINS IN THE ASSESSMENT OF STATE OF NUTRITION IN POPULATION GROUPS.

(Tara Kundaji)

The present work was carried out in order to evaluate the different methods of estimation of blood proteins and to evolve a rapid clinical method for the estimation of blood proteins, in the assessment of state of nutrition in large population groups. The table below gives an idea of the observations made by using some of the methods detailed in our previous report.

	No. of samples analysed.	Average total protein conc. per 100 ml.	Per cent. diff. from Kj. method (No. 6).	Average Sp. Gr. of Serum
1. Phillips and Van Slyke CUSC ¹ method ...	225	6.88	9.76	1.0254
2. Moore and Van Slyke direct specific gravity method.	21	6.82	3.53	1.0268
3. Linderstorm-Lang "density gradient method".	15	6.23	11.88	1.0250
4. Barbour and Hamilton "Falling drop method".	45	6.41	7.92	1.0258
5. Folin and Wu Colorimetric method ...	30	6.93	2.0	
6. The Standard Micro-kjel-dahl method ...	225	7.07		

The results obtained by the CusO₄ method of Phillips *et al* were statistically satisfactory. Certain drawbacks observed in this method were overcome by making suitable modifications. All the other methods require cumbersome apparatus and skilful handling. Hence they are not applicable in the field. This modified method is the simplest and the most rapid of all the methods, and suitable for application in the field. This method requires further investigation.

XXIII. THE INFLUENCE OF CHOLINE, METHIONINE AND CYSTINE ON THE EXCRETION OF URINARY NITROGEN IN RATS.

(Govind and Radhakrishna Rao)

12 young healthy male albino rats, 80-95 gms in weight, were equally distributed into 4 groups, with respect to weight and littermates and kept on a high fat-low protein basal diet, with the necessary supplements of Vitamin A, Vitamin D and Vitamin B complex. Group I animals were given no other supplements whereas groups II, III and IV received respectively 100 mg. per cent. choline, 100 mg. per cent. methionine and 100 mg. per cent. cystine for the first 15 days. Later for the next 6 days, Group II animals received 250 mg. per cent. of choline, Group III animals received 250 mg. of methionine and Group IV animals were given 100 mg. per cent. of choline in addition to 100 mg. per cent. of cystine. The results of urinary nitrogen excretion per period (of three day each) is given in table below:—

Group.	Period.							Total excretion in the first 5 periods.
	1	2	3	4	5	6	7	
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1	50.8	44.8	41.0	42.3	39.6	24.9	29.9	218.6
2	38.5	37.8	26.3	30.8	33.6	39.4	34.0	177.0
3	18.4	26.7	18.2	19.0	20.6	28.0	19.6	102.5
4	46.9	35.9	25.7	21.5	30.3	25.0	26.0	164.3

The results of analysis of the first 5 periods (when the animals were given lower levels of supplements) show clearly that all the supplements at the levels used have a urinary nitrogen sparing action. It is also found that methionine has it to a higher degree than choline, and choline to a slightly lesser extent than cystine. The increase of choline from 100 mg. per cent. to 250 mg. per cent. showed an increased excretion of urinary nitrogen than the controls, whereas addition of choline to cystine, or increase in methionine level did not show any such activity.

XXIV. INFLUENCE OF INOSITOL ON THE NITRO METABOLISM OF THE RAT.

(Govind and Radhakrishna Rao)

Four groups of rats each consisting of three animals, were placed on a high fat-low protein diet with the necessary supplements and in addition they were given supplements of inositol as follows:—

Group (1)—Basal diet alone, Group (2)—B.D. plus 25 mg. per cent. inositol Group (3)—B.D. plus 100 mg. per cent. inositol and Group (4)—B.D. plus 250 mg. per cent. inositol. Nitrogen balance studies reveal

that inositol at the various levels supplied do not show any sparing effect on urinary or faecal nitrogen excretion, as is obtained with choline, methionine or cystine supplementation.

The results of the analysis of the liver for its fat content showed that with higher amounts of supplement of inositol, the level of fat in the liver was lower than that in the control group, nevertheless, the influence of inositol as a lipotropic factor is not so marked.

XXV. KEEPING QUALITIES OF SEMPLE'S VACCINE.

(Dogra)

Two more brews were subjected to repeated assays with the results similar to those reported last year. These findings along with the previous ones, are tabulated below :—

LD50 Potency Value of Semple's Vaccine stored at room temperature in Bombay and in a refrigerator (5-8°C).

Period of storage.	Brew No 77/49		Brew No. 140/49		Brew No. 822/49	Brew No. 825/49	Remarks
	Room	Cold	Room	Cold	Cold	Cold	
4 wks. ...	...	...	...	...	72,440	72,440	Initial potency of vaccine when 2 wks. old was as under :—
6 wks. ...	...	...	...	...	33,110	10,240	
8 wks. ...	1,445	43,650	1,778	46,770	100,000	61,660	
16 wks. ...	75	11,750	0	4,467	...	...	
20 wks. ...	...	...	...	...	31,620	34,670	
24 wks. ...	...	14,790	...	14,130	...	...	77/49 2,512
32 wks. ...	...	1,950	...	9,333	74,130	58,880	140/49 22,910
40 wks. ...	...	562	...	7,943	57,540	42,660	822/49 19,500
48 wks. ...	...	...	...	724		...	825/49 33,110

This work is in hand. The above data suggest (a) that Semple's Vaccine rapidly deteriorates if not stored in the cold; (b) that it "matures" on storage, and (c) that it retains its potency on cold storage to a much longer period than the accepted 6 months, depending probably on the initial titre.

XXVI. ANTIGENIC PROPERTIES OF PHENOL-KILLED RABIES VACCINE PREPARED WITHOUT INCUBATION AT 37°C.

(Dogra).

Heat adversely affects the antigenic properties of Rabies Vaccine. In Semple's method of preparation of Rabies Vaccine 24 hour exposure to heat at 37°C is required. Two batches of vaccine were made in which incubation at 37°C was not resorted to. Paris strain of rabies fixed virus was harvested in sheep. The brain was dissected and macerated with Waring Blendor. A 10 per cent. suspension was made to which USP Acid Carbollic was added in the proportion of 1 in 100; kept at 70°F for 24 hours, filtered and diluted to make 5 per cent. suspension - with 0.5 per cent.

phenol; stored for 30 days. In this vaccine inactivation of the virus occurred in 1 to 2 weeks and the mouse potency test (Habel's technique) showed a high potency level. Brew No. 860/49 and 39/50 gave 134,900 and 91,200 LD 50 protective values respectively.

Vaccine made from the same brain showed considerable difference in the antigenic property, when prepared by Semple's method and when heat was not used and the vaccine prepared as described above. An eight weeks' old Semple's vaccine was assayed along with an eight weeks old non-incubated vaccine. The potency values were 28,840 and 104,700 respectively. The difference, it is suggested, was due to exposure of vaccine to heat of incubation at 37°C for 24 hours, as all other conditions were the same for both vaccines.

XXVII. MECHANICAL MACERATION OF SHEEP BRAIN IN PREPARATION OF RABIES VACCINE.

(Dogra).

Microscopic studies of brain substance showed that with Waring Blendor the nerve tissues did break up but not entirely. Stained smear preparation showed whole nerve cells with their entire structure in tact. For further breaking up of the nerve tissues with a view to aid liberation of rabies virus mechanical aids were tried.

(a) Compressed air or electrically operated stirrers were used. These were unsuitable as contaminations occurred despite all care.

(b) Mechanical shaker was successful but this method was not considered suitable for large-scale production of vaccine.

(c) *Milling*.—It was found that an electrically operated ball-mill could very effectively break up the brain substance. Then details regarding the type, size and shape of the containers and the type and size of the balls were worked out. One litre Pyrex Roux bottles with a rubber stopper and 3/8" hard glass beads were found most satisfactory for purposes of milling sheep brain. The optimum period of milling was found to be two hours.

XXVIII. INACTIVATION OF FIXED RABIES VIRUS WITH MILLING AND PHENOL.

(Dogra).

It was observed that milling not only caused breaking up of brain substance but also enabled the inactivating substance to come into closer contact with the virus and thus affect inactivation of the virus in a shorter period. Several experiments were conducted. The results are summarised below :—

(i) A 10 per cent. brain suspension with 1 per cent. phenol could be inactivated in 5 to 7 days after a 20 to 24 hours of milling. Inactivation occurred within 24 hours if incubated for 24 hours at 37°C, in addition.

(ii) A 20 per cent. brain suspension was not inactivated with 1 or 1.5 per cent. phenol if milled up to 4 hours, but, it was inactivated within half an hour if milled with 2 per cent. phenol.

Further work is in progress with milled vaccine.

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