



सत्यमेव जयते

REPORT
OF
THE HAFFKINE INSTITUTE

For the Year 1939

BY

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BOMBAY

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1939.

PART I.

STAFF.

Director.

Lieut.-Colonel S. S. Sokhey, M.A., M.D. (Edin.), D.T.M. & H. (Eng.),
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I.M.S.

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(Liverpool).

Supernumerary Officer.

Major S. S. Bhatnagar, M.B.B.S. (Pb.), M.R.C.P. (Lond.), M.R.C.S.
(Eng.), Ph.D. (Lond.), from 24th to 31st December 1939.

Department of Pharmacology.

Dr. B. B. Dikshit, Ph.D. (Edin.), M.R.C.P. (Edin.), D.P.H. (Cal.).

Dr. B. K. Nandi, Ph.D., M.Sc.

Department of Bio-chemistry.

Mr. M. A. Malandkar, M.A., M.Sc.

Mr. S. K. Gokhale, B.A., M.Sc., A.I.I.Sc.

Department of Entomology.

Dr. M. Sharif, D.Sc. (Pb.), Ph.D. (Cantab.), B.M.S. (I).

Diagnostic Department.

Mr. N. P. Dalal, M.B.B.S. (Bom.), D.P.H., D.T.M. & H. (Eng.), B.M.S. (I).

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Mr. T. K. Puduval, B.A.

Mr. A. S. Narsimham, B.A.

Mr. R. Sadashivan, B.Sc.

Mr. M. K. Habbu, B.Sc.

Anti-rabic Department.

Mr. R. Sanjiva Rao, M.B.B.S. (Mad.), D.T.M. (Liver.), B.M.S. (I).

Mr. R. K. Gokhale, L.C.P. & S., S.M.S.

Anti-plague Inquiry.

Dr. D. L. Shrivastava, D.Sc.

Mr. R. G. Sathe, G.B.V.C., Veterinary Assistant Surgeon.

Voluntary Research Workers.

Dr. R. Row, O.B.E., M.D., D.Sc. (Lond.).

Dr. S. N. Gore, L.M. & S. (Bom.).

Mr. M. Sadashiv Rao, M.A.

Mr. K. Ganapathi, M.Sc.

Office Staff.

Mr. C. R. Deshpande	.. Head Clerk.
Mr. D. S. Dixit	.. Storekeeper.
Mr. D. N. Mhaskar	.. Accountant.
Mr. S. J. Narvekar	.. Stenographer.
Mr. M. V. Kamat, B.A.	.. Librarian.
Mr. R. D. Panwalkar, A.M.	.. Artist and Photographer.
Mr. D. S. Mondkar	.. Engineer.
Mr. M. K. Patel	.. Packing.

Junior Staff, Subordinates and Inferior Establishment number 120.

GENERAL REVIEW.

The year under review did not see any radical changes in the organisation or in the activities of the Institute. As in previous years the Institute produced large quantities of plague prophylactic and other vaccines and carried out diagnostic work for hospitals and private practitioners. The quantities of vaccines produced were:—Haffkine Plague vaccine 2,887,527 c.c., Cholera vaccine 404,798 c.c., Typhoid vaccine 17,207 c.c. and Anti-rabic vaccine, 5 per cent suspension, 733,805 c.c. Research activities of the Institute detailed elsewhere in the report were aided by a grant of Rs. 50,000 from the Indian Research Fund Association. Lady Tata Memorial Trust gave two scholarships for research work at the Institute.

During the year a vacancy occurred on the staff of the Institute through the retirement of Lieutenant Hugh Maurice, I.S.O., I.M.D., after long and meritorious service. In his place, services of Dr. Sanjiva Rao were secured. This officer was specially selected for his special training and work on Virus diseases. This department of studies has gained great importance during recent years and the appointment of Dr. Rao should prove of great value to the Institute.

PLAGUE DEPARTMENT.

(1) *Chemotherapy of Plague*.—Researches into the chemotherapy of plague have yielded very important results and beget the hope that an effective remedy for bubonic plague is within sight. During the year syntheses of new sulphonamide compounds were undertaken at the Institute by Mr. K. Ganapathi, a research chemist endowed by the Lady Tata Memorial Trust. Of the 35 compounds prepared six were tested for their action on mice which had been experimentally infected with plague. One of these, Sulphathiazole, the synthesis of which had also been reported by the workers abroad, was found to show a very remarkable power to cure plague. When given in adequate dosage this drug was found to save 80 to 90 per cent of the plague infected mice, which have a mortality of 100 per cent if they are not treated. Another well known sulphonamide compound, Sulphapyridine (M & B 693), was tested at the same time and it was found that it also had considerable curative action but only if it was given very early in the course of the disease. If the administration of this drug was delayed until 72 hours after the induction of infection, it saved no more than 10 per cent of the infected mice, while Sulphathiazole still saved 80 per cent. Thus in animal experiments Sulphathiazole proved to be much superior to Sulphapyridine (M & B 693).

At the time of writing the results are also available of a field trial on human cases carried out at Bettiah, Bihar, with the co-operation of the Bettiah Raj. A detailed account of this trial will be given in the next year's report. But the general trend of results may with advantage be indicated here because of their immediate importance. During this trial Haffkine Institute Antiplague Serum, Sulphapyridine and Sulphathiazole were tested for their curative action in plague. No

selection of cases was made for a particular kind of treatment, admissions in rotations were given different treatments. But because the different treatments were brought into use at different stages of the field trial the number of patients in each group did not work out to be equal. Two hundred and thirty-seven cases were dealt with as follows :—

All cases of Bubonic Plague treated.

Treatment.	Number of cases.	Deaths.	Case mortality per cent.
Antiplague Serum	70	20	28·5
Sulphapyridine	53	13	24·5
Sulphathiazole	32	5	15·6
Controls—treated with iodine solution intravenously.	82	43	52·4
Total ..	237	81	

During the field trials it was noticed that the most important single factor which decided the issue in human plague was the development of septicæmia. If the lymph glands prevented the spread of the infection to the blood stream and the infection remained localised spontaneous recovery usually resulted. On the other hand, if the organisms passed the lymph-glands and septicæmia resulted death invariably followed unless an effective curative agent was given to control the infection. Therefore, a truer picture of the results is presented if only those cases are considered in which septicæmia was present at the time the treatment was started.

Plague cases with plague septicæmia at the commencement of the treatment.

Treatment.	Number of cases.	Deaths.	Case mortality per cent.
Antiplague Serum	33	20	60·6
Sulphapyridine	30	13	43·3
Sulphathiazole	12	5	41·8
Usual hospital Iodine Treatment (Controls) ..	40	38	95·0
Total ..	115	76	

It will be noted that in human cases when septicæmia had set in before the administration of the drugs was commenced, the action of both Sulphathiazole and Sulphapyridine is about the same; both saved about 60 per cent of the cases. In the case of mouse infection, under similar conditions, Sulphathiazole proved to be much more effective. Sulphathiazole saved about 80 per cent of the mice with blood infection while Sulphapyridine saved only about 10 per cent. These results are contrary to expectation, because plague infection in mice is much more severe than in human beings. Hundred per cent of the infected mice always die if not treated while in human epidemics case mortality seldom exceeds 80 per cent. and may be as low as 50 per cent. and in the present case it was only 52·4 per cent. It was, therefore, expected that the drug would show much greater curative action in human infection. The explanation of this apparent anomaly seems to be that both the drugs were administered to human cases in inadequate dosage; after an initial dose of 1 g. (two tablets), the drug was continued in 0·5 g. dosage every 4 hours for seven days. If however the temperature of the patient became normal earlier the drug was stopped. It seems certain that the dosage employed was insufficient. It is proposed to employ these drugs in a much larger dosage in the next field trial.

In spite of the inadequate dosage, the curative results are remarkable. Further, these sulphonamide drugs have many advantages over the serum. The drugs are administered by the mouth, are comparatively cheap to make and do not deteriorate on storage. While Anti-plague Serum is expensive to produce, has to be administered by the intravenous route and deteriorates very rapidly unless stored in refrigerated space. In India where plague is now almost entirely a rural problem the sulphonamide drugs have every thing in their favour. Further, and this is more important, the same drugs are equally effective in a number of other infections, such as pneumonia and blood poisoning.

Synthetic organic chemistry has during recent years yielded compounds of the greatest value to medicine. It is certain that this particular branch of research is destined to become even more important in the near future, particularly the chemotherapy of bacterial diseases. There is a great deal of chemical talent available in India, but chemists working in isolation by themselves cannot achieve much. For worthwhile work intimate collaboration of chemists, bacteriologists and pharmacologists is essential. The Haffkine Institute is admirably suited as centre for such collaboration. It would be a great gain if a permanent department of chemotherapy is organised at the Institute.

(2) *Plague Vaccine*.—The study of physical conditions, the temperature of incubation and the duration of incubation under which the most

effective plague vaccine can be produced, was continued during the year. It was also found that the specific immunogenic portions of the plague organism go into solution when the organism is grown in broth or when agar growths are suspended in water. It seems that the rejection of the bacterial debris from both the broth growth vaccines and the agar grown vaccines would yield decidedly less toxic but equally effective vaccines.

(3) *Serology of Plague*.—Carefully conducted agglutination and agglutination absorption tests failed to show any serological difference between the various local strains of *P. pestis* studied. No serological difference was detected even between the virulent and avirulent forms of the organism.

(4) *Epidemiology of Plague*.—Studies started some years ago were concluded and the results are being embodied in a paper for publication. From 1931 to 1936, a steady reduction in the susceptibility to plague infection of Bombay rats (*Rattus rattus*) was observed. The mortality resulting from the standard infective dose declined from 9·3 per cent to 0·0 per cent. During the same period the number of plague infected rats found in the City of Bombay during the routine examination of about 200,000 rats yearly, declined from 748 to 0. Since 1936 no plague infected rats have been found in the City. Because of the large number of rats examined it may be taken that plague epizootic among rats in the City has entirely come to an end. Under the conditions it was interesting to observe that the susceptibility to plague infection of the house rat in the City has begun to increase, as indicated by the increase in mortality from standard plague infective dose from 0·0 per cent in 1936 to 10·0 per cent in 1939. These observations indicate that with the dying out of the epizootic the susceptible strains of rats have begun to multiply again. This fact coupled with the other tendency observed that a highly susceptible species, *Funomys varius*, is gradually invading the City, and is now forming something like 40 per cent of the rat population indicates that the position of the City as regards the possible recrudescence of plague is worsening.

(5) *Metabolism of the Plague Bacillus*.—A study of the nutritional requirements of the organism has shown that three amino-acids—proline, phenylalanine and cystine—are necessary for growth in a chemically defined medium, and that more complex growth factors such as thiamin, nicotinic acid, etc., are not indispensable. These latter factors seemed to have a stimulatory effect in the growth and multiplication of the organism.

DEPARTMENT OF PHARMACOLOGY.

The nature of the destruction of plasmoquine by the body tissues was investigated further and it was shown that the agents responsible for its destruction were probably enzymes and the organs chiefly concerned in order of their activity were, brain, liver, spleen, testis and kidney.

The destructive product was isolated and its pharmacology was studied. Researches into the action of atebirin in monkey malaria showed that the drug did not act directly on the parasites but through the mediation of reticulo-endothelial cells. It was also observed that plasmoquine stimulated the oxygen uptake of body tissues.

During the year there was an increased demand for the services of this department for the biological assay of drugs. This work the department cannot very well undertake as it is a purely research department maintained by the Indian Research Fund Association. Further as legislation was recently passed to control the preparation and the sale of drugs, the Province will have to establish a Pharmacological Department to carry out this control effectively. Such a department can be organised most effectively at the Institute because of the facilities already existing there. The department will have to carry out the following types of work :—

- (1) Bio-assay Section.
- (2) Chemical Section.
- (3) Serum Testing Section.
- (4) Vitamin Section.

Besides carrying out analyses for testing the purity and strength of drugs for control work, the new department will discharge another even more valuable function. Strict control of the manufacture and sale of drugs will give an impetus to the production of drugs in the Province itself. At the start, and perhaps for some considerable time to come, the manufacturing firms will not be able to afford highly developed scientific departments of their own for final exact testing of the products. This aid the Institute can provide quite inexpensively. The department will thus by testing and advice promote the development of the industry.

DEPARTMENT OF BIOCHEMISTRY.

Besides the routine diagnostic work for hospitals and private practitioners, the department continued its studies of the blood urea clearance test. One hundred normal Indian male subjects were examined. The average values obtained were 44 c.c. for maximum clearance and 34 c.c. for standard clearance. The corresponding averages for Americans were 75 c.c. and 54 c.c. respectively. This is a most useful set of observations for clinical work. McIntosh and Van Slyke's blood urea clearance test is now very commonly used in India for determining renal efficiency in diseased conditions and if the American figures are relied upon the results obtained would be very misleading.

The department has also been working to prepare basal metabolic standards for use in India. This was made imperative by the studies carried out in the department which showed that the B.M.R. for Indians was lower than that for Americans by about 8 per cent. The use of American standards for evaluating B.M.R. for Indians was consequently misleading.

DEPARTMENT OF ENTOMOLOGY.

Investigations into the effects of different constant temperatures and humidities on the rate of growth of early stages of the three common Indian rat-fleas of the genus *Xenopsylla* were carried out. As the increase and decrease of flea population during different seasons of the year is a factor of extreme importance in the spread of plague, these studies are filling the gaps in our knowledge of the epidemiology of plague.

DIAGNOSTIC DEPARTMENT AND THE DEPARTMENT OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY.

Over 12,000 samples were examined by the department for various hospitals and private practitioners. Free aid to private practitioners in the diagnosis of suspected cases of typhoid fever was continued during the year. On the receipt of a request from any private practitioner the Institute sends out an officer to take blood and other samples from a patient suspected of typhoid infection. Five hundred and seventy-five calls were answered during the year and 256 cases were diagnosed as suffering from typhoid infection. This free aid to private practitioners has proved very valuable in the treatment and proper notification of cases of this infectious disease. Proposals are before Government to extend this free aid in the case of other infectious diseases also.

ANTIRABIC DEPARTMENT.

The policy of decentralising antirabic treatment was continued and 14 new centres were opened making a total of 101 centres in the Province. The easy availability of antirabic treatment near the patients' homes is making the treatment popular. It is hoped that more centres will be opened in the near future.

SOCIAL LIFE AT THE INSTITUTE.

The Critics Club has had a very active life. It now meets once a week and affords more opportunities to the members of the staff to present and discuss the work being carried out by them. These meetings are also being utilized to consider recent advances in knowledge in the various subjects of interest to the workers at the Institute.

During the year the Club gave farewell parties to two members of the staff, Rao Bahadur G. D. Chitre and Lieutenant Hugh Maurice on their retirement. Rao Bahadur G. D. Chitre had had the longest connection with the Institute. He began as member of the original British Plague Commission in 1908 and kept up his studies into the epidemiology of plague right through his service. Lieutenant Hugh Maurice also had had a long service at the Institute during which he had organised and made a great success of the Antirabic Department. Both officers were universal favourites for their personal as well as professional qualities. The Club presented them with suitable mementoes.

Haffkine Institute Co-operative Credit Society Limited.—The Society has continued to grow and prove of considerable value to its members. It has been a great boon to the lower paid staff.

SECTION I.

VACCINE AND SERUM DEPARTMENT.

A. Haffkine's Plague Vaccine.

962,509 doses (i.e. 2,887,527 c.c.) of plague vaccine were issued during the year against 1,137,086 doses in the previous year.

The number of doses of plague vaccine despatched monthly during the years 1938 and 1939 are detailed below :—

					1938.	1939.
January	..	..	..	..	190,419	51,350
February	..	..	..	..	78,931	104,138
March	..	..	..	..	109,280	115,942
April	..	..	..	..	6,170	58,618
May	..	..	..	..	5,390	4,910
June	..	..	..	..	570	6,660
July	..	..	..	..	46,974	17,303
August	..	..	..	..	84,459	121,323
September	..	..	..	..	234,889	169,267
October	..	..	..	..	100,027	110,171
November	..	..	..	..	99,341	90,153
December	..	..	..	..	180,636	112,674
Total ..					<u>1,137,086</u>	<u>962,509</u>

From the opening of the laboratory to the end of 1939 a total of 44,334,303 doses have been issued. The output of the vaccine in quinquennial periods has been as follows :—

Years.				Doses.
1897-1901	..	..	..	2,867,041
1902-1906	..	..	..	3,455,537
1907-1911	..	..	..	3,584,262
1912-1916	..	..	..	4,567,366
1917-1921	..	..	..	6,667,894
1922-1926	..	..	..	7,202,636
1927-1931	..	..	..	5,929,243
1932-1936	..	..	..	6,636,392
1937-1939	..	..	..	3,423,932
Total ..				<u>44,334,303</u>

The geographical distribution of the vaccine in India and to places outside India during the years 1938 and 1939 is given below :—

				Number of doses.	
				1938.	1939.
BRITISH INDIA.					
Bombay Province	..	..	..	59,436	129,780
Madras Province	..	..	..	139,802	36,762
Bengal, Bihar and Assam	..	..	..	30,283	47,946
United Provinces of Agra and Oudh	..	..	..	231,180	183,360
Punjab	..	..	..	3,098	2,294
North West Frontier Province	..	..	..	292	300
Central Provinces and Berar	..	..	..	39,812	41,786
Delhi Province	..	..	..	489	92
Sind	..	..	..	8	324
Port Blair	..	..	..		24
Total ..				504,400	442,668
INDIAN STATES.					
Southern Maratha States	..	..	..	17,934	28,756
Hyderabad Deccan	..	..	..	124,940	226,800
Mysore	..	..	..	257,730	107,736
Patiala	..	..	..		30
Kashmir	..	..	..	1	
Jaipur	..	..	..		47
Jodhpur	..	..	..		60
Bhopal	..	..	..	2,400	
Indore	..	..	..	16	30
Cochin	..	..	..	504	
Rewa	..	..	..	1,428	
Baroda	..	..	..	1	
Gwalior	..	..	..		600
Junagad	..	..	..		1,800
Benares	..	..	..	3,504	5,157
Jammu	..	..	..	1,800	
Travancore	..	..	..	504	
Bikaner	..	..	..	504	
Rajputana	..	..	..		36
Panna	..	..	..		75
Tikamgarh	..	..	..		3,696
Total ..				411,266	374,823

				Number of doses.	
				1938.	1939.
FOREIGN COUNTRIES.					
Burma	..	..	..	219,630	143,052
Aden	..	..	..	1,008	
Federated Malay States	..	..	..	590	744
Cairo (Egypt) -	..	..	..		1,200
Muscat (Arabia)	..	..	..		20
Portuguese Government	..	..	..	192	
French Government	..	..	..		2
Total ..				221,420	145,018
Total number of doses ..				1,137,086	962,509

B. Cholera vaccine issued month by month from 1937 to 1939.

Month.				1937. in c.c.	1938. in c.c.	1939. in c.c.
January	..	..	..	7,958	4,165	12,882
February	..	..	..	5,650	2,352	11,124
March	..	..	..	11,688	8,421	2,525
April	..	..	..	33,655	924	2,720
May	..	..	..	87,339	31,120	110,037
June	..	..	..	41,235	235,938	97,349
July	..	..	..	207,945	96,692	84,726
August	..	..	..	135,100	85,755	53,611
September	..	..	..	49,360	93,065	11,820
October	..	..	..	19,453	20,019	9,540
November	..	..	..	11,168	15,925	3,119
December	..	..	..	30,427	14,130	5,345
Total ..				640,978	608,506	404,798

C. Other Vaccines.

Statement of vaccine issued monthly during the year 1939.

Month:				Mixed T.A.B. Vaccine in c.c.	Meningo- coccal Vaccine in c.c.
January	..	..	..	256	15
February	..	..	..	292	4
March	..	..	..	579	10
April	..	..	..	665	50
May	..	..	..	647	3,210
June	..	..	..	2,991	
July	..	..	..	2,773	
August	..	..	..	2,630	
September	..	..	..	2,310	
October	..	..	..	3,211	
November	..	..	..	287	
December	..	..	..	566	
Total ..				17,207	3,289

D. *Sera.*

During the year under report the following sera were issued by the Institute :—

	Ampoules.
1. Anti-Tetanic Serum (Prophylactic) 1,500 International Units, 1½ c.c.	10,569
2. Anti-Tetanic Serum (Curative) 20,000 International Units, 10 c.c.	2,140
3. Anti-Diphtheria Serum (Prophylactic) 1,000 International Units, 1 c.c.	256
4. Anti-Diphtheria Serum (Curative) 10,000 International Units, 10 c.c.	367
5. Anti-Dysenteric Serum 8,000 International Units, 20 c.c.	817
6. Anti-Streptococcus Serum Polyvalent. 10 c.c.	179
7. Anti-Meningococcus Serum, 10 c.c.	77
8. Anti-Gas Gangrene Serum, 10 c.c.	1,157
9. Normal Horse Serum 5 c.c.	43
10. Normal Horse Serum 10 c.c.	475

SECTION II.

EPIDEMIOLOGY OF PLAGUE.

This department carried out a number of routine duties and researches during the year.

A. *Examination of Bombay City Rats.*

The examination of rats and other rodents of Bombay City, for evidence of plague infection, was continued during the year. A permanent establishment is maintained by the Municipality of Bombay for catching rats and an average of 2,000 rats is caught every night in the City. These rats are received in the Institute marked with the locality from which they are caught by means of labels tied to their tails. The rats which arrive dead at the Institute are dissected and examined for post-mortem signs of plague. In doubtful cases smears and cultures are also made.

In the detection and control of plague in the City this work is calculated to give the municipal authorities, as it were, the first alarm signal of any plague epizootic that may possibly take place in any particular part of the City ; besides providing valuable information on the subject of epidemiology.

Tables summarising the monthly figures of rats received and the number out of them examined are given below (Table Nos. I to IV). During the year no plague infected rat was detected and no human case of plague occurred in the City.

TABLE I.

Rats received at the Institute from the Municipality during 1939.

Months.				Alive.	Dead.	Total.
January	..	..	..	37,235	33,437	70,672
February	..	..	..	32,922	27,326	60,248
March	..	..	..	34,803	30,230	65,033
April	..	..	..	33,167	24,136	57,303
May	..	..	..	32,119	25,194	57,313
June	..	..	..	33,887	22,775	56,662
July	..	..	..	40,785	25,208	65,993
August	..	..	..	43,152	26,640	69,792
September	..	..	..	37,307	24,508	61,815
October	..	..	..	46,965	28,698	75,663
November	..	..	..	37,804	27,839	65,643
December	..	..	..	41,277	33,333	74,610
Total ..				451,423	329,324	780,747

TABLE II.

Examination of Dead Rats for Plague during 1939.

Months.				Dead rats examined.	Found plague infected.	
					Rattus norvegicus.	Rattus rattus.
January	..	..	..	21,838	} Nil.	} Nil.
February	..	..	..	15,239		
March	..	..	..	16,885		
April	..	..	..	14,455		
May	..	..	..	17,519		
June	..	..	..	15,074		
July	..	..	..	16,680		
August	..	..	..	18,308		
September	..	..	..	14,192		
October	..	..	..	20,820		
November	..	..	..	18,614		
December	..	..	..	17,606		
Total ..				207,230		

TABLE

Table giving in monthly figures of rats trapped alive by Municipal

Species.	January.		February.		March.		April.		May.		June.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus</i> ..	7,411	25·2	5,566	22·1	4,737	19·2	5,773	23·9	6,429	23·5	4,821	17·8
<i>Rattus norvegicus</i> ..	7,719	26·3	7,048	28·0	6,463	26·3	6,464	26·7	7,000	25·6	6,288	22·7
<i>Gunomys varius</i> ..	8,770	29·8	6,583	26·1	8,285	33·6	7,885	32·6	10,259	37·5	11,671	43·2
<i>Bandicota indica</i> ..	373	1·4	394	1·6	366	1·5	306	1·3	208	0·8	370	1·5
<i>Mus musculus</i> ..	1,638	5·7	1,449	5·7	1,291	5·2	1,204	5·0	1,087	4·0	1,029	3·9
<i>Crocidura cœrulea</i> ..	1,857	6·4	1,314	5·2	1,261	5·1	1,697	7·0	1,970	7·2	2,546	9·5
*Unidentifiable ..	1,720	5·2	2,847	11·3	2,242	9·1	845	3·5	371	1·4	362	1·4
†Total ..	29,488	100·0	25,201	100·0	24,650	100·0	24,174	100·0	27,324	100·0	27,087	100·0

* Rats included under this head though reported to have been caught alive, were received at the Institute dead.

† These totals do not agree with the totals of live rats in Table I because rats received on Sundays and holidays

III.

Corporation, Bombay, during 1939—classified according to species.

July.		August.		September.		October.		November.		December.		For whole period.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
6,153	17·7	7,719	21·3	5,891	22·1	14,847	37·2	8,321	27·2	6,638	23·9	84,311	23·8
8,445	24·2	8,560	23·5	6,300	23·6	8,053	20·2	7,014	23·0	7,109	25·5	86,468	24·4
15,006	43·0	14,914	41·1	10,474	39·2	12,040	30·2	10,250	33·5	9,524	34·3	125,661	35·5
537	1·5	396	1·1	253	0·9	282	0·7	449	1·5	377	1·4	4,311	1·2
1,051	3·0	1,621	4·5	1,255	4·7	1,546	3·8	1,769	5·8	1,380	5·0	16,320	4·6
3,362	9·6	2,808	7·7	2,406	9·0	2,909	7·3	2,163	7·1	2,037	7·3	26,330	7·5
329	1·0	302	0·8	142	0·5	239	0·6	576	1·9	735	2·6	10,710	3·0
31,888	100·0	36,320	100·0	26,721	100·0	39,916	100·0	30,542	100·0	27,800	100·0	354,111	100·0

often mutilated and even putrid,
were not classified.

TABLE

Table giving the classification of rats founded

Species.	January.		February.		March.		April.		May.		June.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
<i>Rattus rattus</i> ..	2,956	11.1	1,757	8.7	2,237	10.6	2,085	12.0	2,267	11.1	1,707	9.7
<i>Rattus norvegicus</i> ..	7,329	27.6	4,863	24.1	5,234	24.7	5,517	31.9	7,387	36.3	6,678	37.7
<i>Gunomys varius</i> ..	6,193	23.4	4,946	24.5	6,119	28.9	5,146	29.7	6,091	29.9	5,506	31.1
<i>Bandicota indica</i> ..	187	0.7	119	0.6	96	0.5	101	0.6	162	0.8	147	0.8
<i>Mus musculus</i> ..	2,412	9.1	1,798	8.9	1,746	8.2	1,205	7.0	1,161	5.7	690	3.9
<i>Crocidura corulea</i> ..	1,806	6.8	1,680	8.3	1,988	9.4	1,686	9.7	2,103	10.3	1,895	10.7
Unidentifiable ..	2,761	10.4	1,756	8.7	1,453	6.8	411	2.4	542	2.7	406	2.3
Putrid ..	2,888	10.9	3,276	16.2	2,320	10.9	1,160	6.7	638	3.2	667	3.8
*Total ..	26,532	100.0	20,195	100.0	21,193	100.0	17,311	100.0	20,351	100.0	17,696	100.0

*These totals do not agree with the totals of dead rats in table No. I because rats received on Sundays and

IV.

dead in the City of Bombay during 1939.

July.		August.		September.		October.		November.		December.		For whole period.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
2,007	9·9	2,136	10·3	1,888	11·6	3,004	12·8	2,489	11·9	2,529	12·5	27,062	11·0
7,020	34·8	7,274	35·0	5,519	34·0	8,248	35·1	7,157	34·3	6,699	33·1	78,925	32·2
6,605	32·7	7,525	36·2	5,711	35·1	7,802	33·3	6,851	32·8	6,345	31·3	74,840	30·6
145	0·7	172	0·8	115	0·7	180	0·8	196	0·9	204	1·0	1,824	0·8
714	3·5	1,045	5·0	865	5·3	1,437	6·1	1,732	8·3	1,696	8·4	16,501	6·7
2,342	11·6	2,037	9·8	1,811	11·2	2,347	10·0	1,703	8·2	1,700	8·4	23,098	9·4
320	1·6	156	0·8	94	0·6	149	0·6	189	0·9	133	0·6	8,370	3·4
1,046	5·2	436	2·1	248	1·5	299	1·3	553	2·7	948	4·7	14,479	5·9
20,199	100·0	20,781	100·0	16,251	100·0	23,466	100·0	20,870	100·0	20,254	100·0	245,099	100·0

holidays were not classified.

B. Examination of Rats caught from

These rats received as they are along with the rats of the other wards of month of April, a separate record of these rats showing the percentage Commissioner with the Government of India. The following Table V

TABLE

Table giving the monthly figures of rats caught alive from

M6h

Species.	April.		May		June		July		Aug
	Num-ber.	Percent-age.	Num-ber.	Percent-age.	Num-ber.	Percent-age.	Num-ber.	Percent-age.	Num-ber.
<i>Rattus rattus</i> ..	201	36·9	318	40·1	271	32·9	416	39·3	313
<i>Rattus norvegicus</i>	87	16·0	87	11·0	87	10·6	109	10·3	62
<i>Gunomys varius</i> .	84	15·5	103	13·0	154	18·7	196	18·5	186
<i>Bandicota indica</i> .	1	0·2	1	0·1	3	0·4	9	0·8	6
<i>Mus musculus</i> ..	122	22·4	245	30·8	250	30·3	258	24·4	289
<i>Oreodura coerules</i> .	39	7·2	30	3·7	43	5·2	70	6·6	60
Unidentifiable ..	10	1·8	10	1·3	16	1·9	1	0·1	1
Total ..	544	100·0	794	100·0	824	100·0	1,059	100·0	917

Rats received on Sundays and

the Bombay Port Trust Docks.

the Bombay Municipality, are included in the daily totals. Since the of different species is also maintained at the instance of the Public Health gives a summary of this record :—

V.

the Bombay Port Trust area from April to December 1939.

thly.

ust.	September.		October.		November.		December.		For whole period.	
Percent- age.	Num- ber.	Percent- age.	Num- ber.	Percent- age.	Num- ber.	Percent- age.	Num- ber.	Percent- age.	Num- ber.	Percent- age.
84.1	354	42.6	489	46.7	383	40.7	379	39.6	3,124	39.5
6.8	47	5.7	86	8.2	83	8.8	60	6.3	708	8.9
20.3	120	14.5	185	17.6	159	16.9	199	20.8	1,386	17.6
0.7	7	0.8	17	1.6	18	1.9	14	1.5	76	0.9
31.5	226	27.2	175	16.7	197	20.9	224	23.5	1,986	25.0
6.5	66	8.0	96	9.2	100	10.6	78	8.2	582	7.4
0.1	10	1.2	..	..	2	0.2	1	0.1	51	0.7
100.0	830	100.0	1,048	100.0	942	100.0	955	100.0	7,913	100.0

holidays were not classified.

C. Examination of Rats from Other Sources.

During the year, 155 rats from different towns of the Province were received and examined and none of these showed any evidence of plague infection. One rat received from a State in the C.P. was found plague infected.

SECTION III.

DEPARTMENT OF PHARMACOLOGY.

Through the generosity of the Indian Research Fund Association, the department was able to continue its activities on the same lines as during the past three years. Dr. B. K. Nandi continued his researches in the chemistry of synthetic antimalarial drugs and synthesized some new compounds of acridine which are being investigated for their chemotherapeutic properties in malarial infections. Dr. Inderjit Singh was awarded a commission in the Indian Medical Service early in the year and as he was posted outside Bombay he had to interrupt his work for a few months. He however was posted back to Bombay and was able to resume his studies on the physiology of muscular contraction. He also investigated the problem of intravenous administration of oxygen in conditions like lobar pneumonia and obtained very encouraging results.

The department was chiefly concerned in the investigation of the pharmacological action of synthetic antimalarial drugs and continued its researches on Plasmoquine and Atebrin.

The fate of Plasmoquine in the body was investigated further and besides confirming the previous observation that the drug is destroyed quickly in the body, the organs which were chiefly responsible for this destruction were identified. A successful attempt was also made to isolate the product of decomposition and pharmacological investigations on this product were carried out. Isolation of the decomposition product was obtained by perfusing the liver of experimental animals with a solution of plasmoquine, and recovering the product by chemical extraction. Studies on the pharmacological action of this decomposition product showed that it differs considerably from original plasmoquine and is considerably less toxic.

Investigations on Atebrin were chiefly directed towards finding its mode of action in monkey malaria. For this purpose the smallest dose of Atebrin required to cure a known degree of infection with *Plasmodium Knowlesi* in monkeys was first determined. By employing this dose of Atebrin it was found that monkeys with their spleens removed and the reticulo-endothelial cells blocked with India ink failed to get cured and a much larger dose was necessary to bring about a cure in such animals. It therefore appears that reticulo-endothelial system plays some role in the curative action of Atebrin.

In vitro experiments with infected blood (blood of monkeys infected with *Plasmodium Knowlesi*) were also made and it was found that a concentration of 1-100,000 of Atebrin was required to kill the parasites

in 24 hours. Such a high concentration is never reached in therapeutic use of the drug in monkeys and thus a direct action of the drug on the parasites was shown to be improbable. Determinations on the atebirin concentration in the blood after therapeutic and even larger doses were made and it was found that the concentration was in the neighbourhood of 1 in 300,000. It was further found that this concentration fails to kill the parasites, if allowed to act in a test tube for 24 hours, thus lending support to the view that the action of Atebrin is not a direct one. Other interesting observation made was the stimulant action of Plasmoquine on tissue respiration. It was found that the oxygen consumption of most of the tissues of the body was increased by Plasmoquine, the red blood cells and the liver being influenced most.

Biological Assay of Drugs.—The number of drugs received for biological assay increased considerably during the year under report. The assay of Tincture Digitalis manufactured by local firms was very much in demand. Besides tincture digitalis, samples of Insulin, Tr. Strophanthus, Ergot extract, Belladonna plaster, etc., were also received for assay. This assistance to manufacturers is proving of great value. Some firms who had their tinctures assayed during the previous years sent fresh samples during the year under report also and it was gratifying to note that the quality of the new samples was much superior to the old ones. Experience of the past few years goes to show that if the manufacturers are told about the quality of their preparation they are quite willing to bring it to the proper standard, if it is not already so. They are only in need of some organisation which will tell them the exact potency of their product.

Miscellaneous Work.—As usual a number of drugs were sent to the department as specifics for snake bite. The department as a rule does not accept any such drug for investigation unless the composition of the drug is revealed and unless the sample is sent by a medical practitioner. It was found necessary to impose these restrictions because of the very large number of drugs sent and also because our extensive previous experience has shown that as a rule such drugs are eventually found to be valueless. Experiments made by us during the past several years number over several thousands and as most of the indigenous drugs were included in this series, it is rare that a new drug is sent. For this reason the composition of the drug is asked for so that we can go over our list and see if the drug has been already tried or not.

SECTION IV.

DEPARTMENT OF BIOCHEMISTRY.

The Biochemical unit has continued to be increasingly useful to hospitals and medical practitioners for the varied biochemical examinations required in the diagnosis of disease. The other departments of the Institute also greatly benefit by the help the unit is able to give in their research work.

1. Routine examinations carried out in the year numbered 1225. A detailed list is given below.

2. 13.3 litres of anti-plague serum were concentrated during the year under report. The method employed was the usual sodium sulphate globulin precipitation one and has been already described in the report of Haffkine Institute, 1932-35, on page 26.

3. Estimations of Vitamin B by an accurate chemical method were carried out as part of the toddy research work.

4. Research work undertaken regarding the study of blood urea clearance of normal Indians has been continued during the year under report. Details are given in Part II.

5. Study of the effect of ammonium on the potassium content of unstriated muscle was carried out.

*Routine examinations carried out in 1939.**Blood—*

Non-protein Nitrogen ..	62
Urea Nitrogen ..	120
Uric acid ..	18
Creatinine ..	21
Cholesterol ..	51
Chlorides ..	10
Sugar ..	73
Phosphorous ..	16
Serum Calcium ..	27
Haemoglobin ..	50
Cell Volume ..	17
Vanden Bergh Reaction ..	15
Plasma Proteins ..	6
Icterus Index ..	6
Plasma (co ₂) capacity ..	1
Colour Indices ..	11
Potassium in serum ..	1
Sodium in serum ..	1

Urine—

General Quantitative test .	95
Total Nitrogen ..	4
Urea Nitrogen ..	64
Uric Acid ..	3
Sugar ..	77
Chlorides ..	3
Albumin ..	30
Ammonia Nitrogen ..	3
H-ion Concentration ..	2
Blood ..	2
Bile ..	7
Lead ..	6
Diastase ..	1
Diazo test ..	1
Urinary Calculus ..	1

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Cerebro-spinal Fluid—

Total Protein	..	89
Globulin	..	1
Sugar	..	87
Chlorides	..	92
Special Protein	..	1
Lange's colloidal gold test	..	31
		301

Faeces—

Fat	..	6
Occult blood	..	11
Pancreatic insufficiency	.	2
		19

Miscellaneous—

Basal Metabolism	..	25
Glucose tolerance test	..	4
Liver function test	..	4
Kidney function test	..	31
Gastric analysis	..	21
Coagulation time of blood	..	2
Leavulose tolerance test.		1
Milk	..	10
Formolgel test	..	1
Fluid, pleuritic	..	1
		100
Total	..	1,225

SECTION V.

DEPARTMENT OF ENTOMOLOGY.

This department was equipped to permit of researches in ecology and morphology of insects especially fleas being carried out. Arrangements were made also to build up a reference collection of insects of medical and veterinary importance in order to afford facilities to public health officers visiting this Institute to know correctly about these insects. This collection of local interest was further enriched by the donation of identified specimens of insects from the following institutes through the courtesy of gentlemen mentioned within the brackets :—

- (1) Malaria Institute of India, Kasauli (Dr. I. M. Puri) ; about 100 specimens of 31 species of mosquitoes.
- (2) Imperial Agricultural Research Institute, New Delhi (Dr. H. S. Pruthi) ; about 50 specimens of 21 species of flies.
- (3) Imperial Veterinary Research Institute, Mukteswar (Mr. S. K. Sen) ; about 20 specimens of 8 species of flies and lice.

During the year several requests about the identification of insects and snakes and general information about insects and their control were received and they were complied with. Identification of fleas of the genus *Stivalius* and information about rearing them and the part they play in the transmission of plague were supplied to Dr. P. C. George, Officer-in-charge of the Cumbum Valley Plague Inquiry. Information about the distribution of *Ornithodoros savignyi* in India was supplied to Professor P. A. Buxton of the London School of Hygiene and Tropical Medicine.

Several samples of rat-traps and a foot pump duster were received during the year for examination and expert advice from different firms and local bodies. Suggestions for improving their construction were given to them. Placards, exhibits, films, etc., on plague and other diseases were supplied to the Health Section of the Poona Industrial Exhibition, 1939, and Professor of Biology, Karnatak College, Dharwar, for public exhibition.

The department was mainly interested in the investigations of effects of different constant temperatures and humidities on the rate of growth of early stages of the three common Indian rat-fleas of the genus *Xenopsylla*. The results of these investigations are summarized in Part II of this report. Further experiments on the nutritional requirements of larvae of these fleas and their bearing on the transference of these fleas from one place to another along with merchandise are in progress.

Training of Health Personnel.

1. Mr. M. A. Unnikrishna Menon, B.A., Entomological Assistant, Public Health Department, Travancore, was given a month's course of training in Medical Entomology with special reference to rat-fleas and plague.

2. Dr. S. Raghavender Rao, L.M.S., D.T.M., D.P.H., Assistant Surgeon, H. E. H. the Nizam's Medical and Public Health Department, Hyderabad, was given facilities to carry on his researches on the epidemiology of plague for three months.

3. Dr. S. Suvi, M.B., Ch.B., D.P.H., Director-Designate of the Vaccine Depot, Meiktila, Rangoon, was given facilities for about a week, to learn the general working of the various departments of the Institute.

4. Dr. V. Narsimhamurthy, B.Sc., M.B., B.S., D.P.H., D.T.M.; Dr. P. H.; Bacteriologist, Public Health Institute, Bangalore, was given a month's course of training in plague work, preparation of vaccine and general laboratory methods.

5. The 1939 class of Sanitary Inspectors was given a short course of demonstrations and lectures about rat-examination and rat—and flea-destruction.

SECTION VI.

DEPARTMENT OF PUBLIC HEALTH, CHEMISTRY AND BACTERIOLOGY.

The following is the résumé of the work done in department during the year under review :—

A. *Chemical Work.*

Waters.—During the year under report 10 samples of water were examined, majority of these being for sanitary purposes. The chemical composition of these waters showed extreme variation in total solids (198–34) and chlorides (62–2) as compared with the recognised chemical standards of purity.

Foods.—Four samples of ghee and three samples of milk were examined for the detection of adulteration.

B. *Bacteriological Work.*

Waters.—During the year under review the following samples were examined :—

Drinking waters	22
Waters from swimming baths .. .	11
	—
Total ..	33

For drinking waters the method outlined in report 71 of the Ministry of Health on the bacteriological examination of waters was followed.

One sample of sausages and one sample of ice-cream were also examined bacteriologically.

C. *Miscellaneous Work.*

Fumigation.—Twenty-nine packages containing plants and bulbs were fumigated for the Customs Department free of charge.

Stains and standard solutions were prepared and supplied to various laboratories of this Province.

The Institute started making 25 per cent. glucose solution for the J. J. Group of Hospitals and from the middle of August up to the end of the year 5½ litres were supplied. This solution is being supplied at the rate of 8 as. per 100 cc. and will prove very economical and of a constant standard purity for the hospitals.

SECTION VII.

DIAGNOSTIC DEPARTMENT.

A. *Bacteriological Section.*

The routine work carried out is summarised in the accompanying table:—

Material examined.	Positive.	Negative.	Total.
<i>A.—Blood—</i>			
(a) Films for microscopical examination—			
For malaria	53	116	169
„ filaria	..	1	1
„ differential counts	..	..	91
(b) Counts for R.B.C.'s	..	..	63
„ „ W.B.C.'s	..	..	55
(c) Cultures for organisms (B. typhosus) ..	157	551	708
(d) Serological examination—			
Wassermann Reaction	1,140	2,441	3,702 (112 doubtful and 9 anticomplementary.)
Kahn test	802	1,850	2,719 (67 doubtful).
Agglutination Tests—			
For enteric group	421	601	1,022
„ undulating fever	1	8	9
„ typhus fever	..	6	6
„ dysentery	1	..	1
Blood grouping	..	..	22
<i>B.—Sputum—</i>			
Microscopical examination for tuberculosis	5	19	24
Cultural examination for tubercle bacilli ..	2	12	14
Cultural examination for various micro-organisms	..	..	8
Animal experiments for tubercle bacilli ..	..	4	4

Material examined.	Positive.	Negative.	Total.
<i>C.—Urine—</i>			
General analysis	...	..	297
Cultural examination	50	76 sterile and contami- nated.	126
Microscopical examination for tuberculosis.	8	30	38
Cultural examination for tuberculosis ..	1	14	15
Cultural examination for typhoid ..	..	16	16
„ „ typhoid carrier ..	..	91	91
Animal experiments for tuberculosis ..	2	10	12
Friedman test for pregnancy ..	4	5	9
<i>D.—Fæces—</i>			
(a) Microscopical examination for amæbæ, cysts and ova.	128	215	343
(b) Cultural examination for dysentery ..	10	34	44
(c) „ „ cholera ..	2	..	2
„ „ typhoid ..	3	46	49
„ „ carrier ..	1	87	88
„ „ miscellaneous organisms.	..	..	31
<i>E.—Cerebro-spinal fluid—</i>			
(a) Microscopical examination for tubercle bacilli and other organisms.	14	82	96
(b) Cell count	..	..	86
(c) Cultural examination	12	80	92
(d) Animal experiments for tubercle bacilli.	..	5	5
(e) Wassermann Reaction	24	81	108 (1 doubtful 2 anti-com- plementary).
<i>F.—Pus—</i>			
(a) Microscopical examination for tubercle bacilli and other organisms.	12	10	22
(b) Cultural examination	33	12 sterile.	45

Material examined.	Positive.	Negative.	Total.
<i>G.—Miscellaneous Microscopical Examinations—</i>			
Smears from swabs for diphtheria ..	1	26	27
Smears for leprosy	68	28	96
„ gonococci	7	43	50
„ Leishman Donovan bodies ..	..	1	1
Smears from gastric fluid for Boas-Oppler bacilli	..	15	15
Smears for tubercle bacilli from pleuritic and other fluids	1	12	13
Smears for leptospira	..	1	1
Smears from chancre for Ducrey's bacilli ..	..	2	2
Smears from chancre for treponema pallidum	..	1	1
Smears from skin scrapings for fungus ..	1	..	1
Microscopical examination of fluid from ovarian cyst for echinococcus hook lets. ..	..	1	1
Miscellaneous fluids for cell count ..	..	..	3
<i>H.—Miscellaneous Cultural Examinations—</i>			
Cultures from throat swabs for diphtheria	2	175	177
„ from swabs for other organisms ..	..	..	27
Miscellaneous fluids such as pleuritic, peritoneal, etc., for pathogenic organisms	7	10	17
Culture for malaria	..	1	1
„ leptospira	..	1	1
Cultures from tooth	..	..	2
Vomits for organisms of Salmonella group ..	..	4	4
Foodstuffs for bacteriological examination ..	..	4 sterile.	4
Catguts for sterility	..	..	9

B. Pathological Section.

During the year under review 568 pathological tissues were received for examination. The following table gives a rough classification of the nature of these tissues and their number :—

	No.	Per cent.
Non-malignant tumours	59	10·4
Malignant tumours	74	13·0
Tissues found to be normal	5	0·9
Miscellaneous pathological conditions (including research specimens).	268	47·2
Received in a state unfit for examination ..	2	0·3
Animal brains for negri bodies	160	28·2
Total ..	568	

Of the 74 specimen malignant tumours subjected to the histological examination 57 were carcinomas, 6 sarcomas, 1 melanoma, 8 epitheliomals 1 seminoma and 1 teratoma.

25 per cent. of epitheliomas and 47 per cent. of carcinomas occurred in the female reproductive organs, cervix of the uterus and ovary being the commonest site, 12 cases of carcinoma of the breast were also diagnosed. Seminoma was confined to the testis and teratoma to the ovary.

Snake Farm.—An open Air Snake Farm was opened for housing the snakes which were formerly kept in metal boxes. The collection of Echis and Krait venoms would enable the preparation of anti-venene against these snakes. The Institute is now in a position to meet freely the requirements of cobra and other snake venoms.

SECTION VIII.**ANTI-RABIC DEPARTMENT.**

During the year 1939, 3,292 persons applied for anti-rabic treatment at the Institute. Out of these 2,960 completed the full course of treatment. 326 were advice cases, as the biting animals which were under observation were alive and well for ten days after having inflicted the bite and found to be not rabid, and six persons discontinued treatment of their own accord.

To date only one death has resulted from hydrophobia.

During the year 7,226 anti-rabic treatments were issued to the centres. These figures include 350 treatments issued for veterinary use.

Fourteen new Anti-rabic Centres were opened during the year bringing the total of out-centres to 101.

- (1) Rao Bahadur Udhavdas Tarachand Hospital, Sikarpur, Sind.
- (2) Civil Hospital, Larkana.
- (3) „ Nawabshah.
- (4) „ Dadu.
- (5) „ Jacobabad.
- (6) „ Mirpurkhas (Sind).
- (7) „ Sukkur.
- (8) Ratlam, Ratlam State.
- (9) Devgad, Baria.
- (10) The State Hospital, Navasari.
- (11) The State Hospital, Patan.
- (12) The State Hospital, Amreli.
- (13) Kurduwadi, Barsi Light Railway.
- (14) Civil Hospital, Pachmarhi.

The details of the treatments carried out in 1938 and 1939 are summarised below :—

Names.	1938.	1939.
Number of persons applied for the treatment ..	3,045	3,292
Advice cases	305	326
Fully treated	2740	2,960
Percentage of health returns	98·8	Awaited.
Cases of hydrophobia	3	1
Mortality rate	0·1 per cent.	
Number of anti-rabic centres	87	101
Number of treatments sent to the centres ..	7,050	6,876
Number of treatments sent to the Veterinary Surgeons.	689	350

Table showing the number of brains received and examined at the Institute for evidence of Rabies during the year 1939.

Details.	Number.	Percentage.
Received in a state unfit for examination ..	10	6.3
No evidence of Rabies ..	37	23.1
Positive for Rabies (Negri bodies present) ..	113	70.6
Total ..	160	

Statement showing the classification of brains and different places from where the brains were received.

Details.	Positive.	Negative.	Unsuitable for examination.	Total.
Received through the Bombay Veterinary College.	33	8	3	44
Received through the Bombay Police ..	4	1	..	5
Received from private owners in Bombay.	2	1	1	4
Received through other places in Bombay.	2	1	..	3
Received through Out-Centres ..	72	26	6	104
Total ..	113	37	10	160

Statement showing the classification of different animals with the results of their brain examinations for rabies.

Animal.	Positive.	Negative.	Unsuitable for examination.	Total.
Dog	105	32	9	146
Cows	2	1	..	3
Buffaloes	1	..	..	1
Horse	..	2	..	2
Bullocks	2	..	..	2
Goats	3	..	..	3
Monkey	..	..	1	1
Cat	..	1	..	1
Human	..	1	..	1
Total ..	113	37	10	160

Statements showing the number of brains received and examined for rabies.

Name.	Number of brains examined.	Positive.	Negative.	Unsuitable for examination.
Bombay City and Suburbs	63	45	12	6
Out-stations	97	68	25	4
Total	160	113	37	10

SECTION IX.

INCOME AND EXPENDITURE.

The following table shows the income and expenditure of the Haffkine Institute for the year 1938-39 :—

Receipts.		Expenditure.	
	Rs.		Rs.
XXVIII-Public Health—		39-D-Public Health—	
Sale proceeds of Sera and Vaccines, etc.	3,10,395	D-Bacteriological Laboratories, Haffkine Institute, and 39-E-Public Health-E-Pasteur Institute—	
Miscellaneous	2,809	Pay of Officers	91,790
		Pay of Establishment	80,802
		Allowances, Honoraria, etc.	8,503
		Contingencies	1,66,765
Total Receipts	3,13,204	Total Expenditure	3,47,860

PART II.

RESEARCH WORK.

I. Plague Vaccine.

(SOKHEY AND HABBU.)

A. *Optimal Temperature of Incubation for P. pestis Cultures.*—In the last report results were reported of 2 to 4 counts made on 48-hour growths in broth at different temperatures. As the experiments were carried out over a period of several months several batches of broth were used and the doubt arose whether significant results were obtained especially as it was observed that even though the composition of the different batches of broth was carefully controlled the growths resulting in them varied. Because of this observation the whole work was repeated. Two sets of experiments were carried out using only two batches of broth. Eight incubators were used at a time maintaining different temperatures and an attempt was made to make six observations at each temperature on each batch of broth. For these experiments the period of growth was limited to 36 hours as against 48 hours in the previous year's experiments. The following results were obtained :—

Temperatures of incubation.					1st batch of broth, 36 hours growth, average counts in millions per cc.	2nd batch of broth, 36 hours growth, average counts in millions per cc.
23°C	..	..	..	..	255	177
25°C	..	..	..	..	258	210
27°C	..	..	..	..	318	240
28°C	..	..	..	..		247
29°C	..	..	..	..	355	261
30°C	..	..	..	..		204
31°C	..	..	..	..	261	174
33°C	..	..	..	..	286	152

A statistical analysis of the figures obtained shows that optimal temperature of growth for *P. pestis* in broth is 27° to 29°C. It is interesting to note that growth at 37°C was very poor. The second batch of broth for a 48-hour period of growth at 37°C gave a count of only 94 millions per cc.

B. Broth and Agar Grown Vaccines.—The difference between the protective power of broth and agar vaccines were studied from still another angle with a view to determining the best method of preparing an effective plague vaccine. Previous observation that a four-week growth in broth incubated at 27°C yielded a more potent vaccine than a similar growth at 37°C was again confirmed. It was also again established that in broth vaccines incubated at 27°C or at 37°C the total protective power resided in the clear fluid of the vaccine and that the bacterial debris had little or no protective power. It was further observed that when 48 or 72-hour agar growths incubated at 37°C are suspended in water, and organisms are left in contact with water for 3 to 7 days and a clear fluid separated from the suspension, the total protective power resided in the clear fluid. But in the case of growths incubated at 27°C, when similarly suspended in water the immunogenic substance did not dissolve readily in the saline i.e. it was found that the clear fluid of the vaccine had very poor protective power.

The explanation of these observations seems to be obvious and present observations seem to strengthen the hypothesis put forward in the Haffkine Institute report for the year 1936, page 37. In the case of the agar growths incubated at 37°C it is clear that the immunogenic substance is present in a soluble form in the organism while it is not so in the case of the growth incubated at 27°C. In the case of the broth growths the immunogenic substance goes into solution both when incubated at 27°C and at 37°C. But as 27°C is the optimal temperature for the growth of *P. pestis*, the growth at 27°C is much more profuse than at 37°C, hence the 27°C grown broth vaccine is more potent. But the potencies of the two vaccines grown at 27°C and 37°C are not directly proportional to the amount of growth at the two temperatures. The reason seems to be twofold. The organism when incubated at 37°C is larger in size than when incubated at 27°C and further at the higher temperature of incubation the organism dissolves more readily. These hypotheses are being studied experimentally. It has been possible to isolate the protein fraction which is responsible for the immunogenic properties of the organism.

This work indicates that only the clear fluid of broth vaccine grown at 27°C or the clear fluid from suspensions of agar growths incubated at 37°C should be used, and the bacterial debris discarded. Such a procedure will not only yield the best possible vaccine, but that such a vaccine would be much less toxic.

II. Field Trial of Anti-plague Serum.

(WAGLE.)

About the close of the year a fresh epidemic of plague was reported from Bettiah, Bihar. A Unit was sent to the place to make further trials of the anti-plague serum. In addition, the action of Sulphapyridine and Sulphathiazole was also studied. A brief note on the results obtained at the time of writing has been given in the general review.

III. Chemotherapy of Plague.

(SOKHEY AND DIKSHIT.)

During the year Mr. Ganapathi, Lady Tata Memorial scholar, took up the synthesis of new compounds, particularly heterocyclic derivatives of Sulphanilamide. An account of this work is given elsewhere in this report. Of the 35 compounds synthesised by him and Dr. Nandi, six were tested in plague infection in mice. Of these Sulphathiazole was found to be possessed of remarkable curative action. The action of this drug was compared against that of Sulphapyridine (M and B 693), the only other drug found to have some action against plague infection. These two drugs were given at four different stages of the infection in mice: (1) at the same time as the infection was given, (2) twenty-four hours after infection, (3) forty-eight hours after infection, and (4) seventy-two hours after infection. It was found that when the administration of the drug was begun before septicaemia had set in, i.e. at the same time as infection and 24 hours after infection, as small a dose of Sulphathiazole as 10 mg. twice a day for ten days saved nearly 80 per cent. of the infected animals. Sulphapyridine produced about the same action as Sulphathiazole in the early stages of infection with doses of the same order. In the second group, when the administration of drug was started after septicaemia had set in, i.e. 48 hours and 72 hours after infection, the remarkable action of Sulphathiazole in curing plague became more evident. A dose of 20 mg. twice a day for ten days cured approximately 80 per cent of infected animals when the drug was given 48 hours after infection and 65 per cent when given 72 hours after infection. Doubling this dose produced even better results, the survivals becoming 90 and 80 per cent respectively, while Sulphapyridine with the largest dose employed, i.e. 40 mg. twice a day for ten days, saved approximately only 10 per cent. of the infected animals in this group.

IV. Epidemiology of Plague.

(SOKHEY AND MENEZES.)

These researches conducted over a period of years have yielded some very important data which have been reported from time to time.

The following table illustrates an important aspect of Epidemiology of Plague.

Year.	Epizootic among rats		Susceptibility of rats to plague	
	Total number of rats examined.	Number found plague infected.	Number of rats infected.	Mortality.
				Per cent.
1931	290,316	748	118	9.3
1932	272,018	600	106	8.5
1933	256,900	393	139	7.9
1934	237,854	34	93	6.5
1935	226,289	0	40	5.0
1936	182,727	0	20	0.0
1937	186,987	0	90	7.8
1938	187,276	0		
1939	207,230	0	50	10.0

From the year 1931 to 1936 a steady reduction in the susceptibility of rats is to be noticed. The percentage mortality resulting from standard infective dose declined from 9.3 to 0.0. After the epizootic had come to an end in 1936, the susceptibility of rats to plague infection began to increase again. It appears that the epizootic was destroying the susceptible strains and after the epizootic had come to an end the susceptible strains have begun to multiply again.

V. A Serological Study of Some Strains of *Pasteurella Pestis*.

(WATS, WAGLE AND PUDUVAL.)

A serological differentiation of representative strain of *P. pestis* was carried out using the technique of agglutinin absorption. The study was made with the 27°C as well as with the 37°C growths. Agglutinable suspensions of the organisms were prepared with 27°C as well as with 37°C growths on acid digest agar. The growth was taken up first in distilled water containing 1 per cent. carbolic acid and later diluted to reduce the concentration of phenol to 0.25 per cent. Sera were prepared by immunising rabbits with dead as well as live culture of *P. pestis*, first subcutaneously with the dead organisms and then intravenously with the live cultures.

Two types of agglutinations were observed, one being specific to room temperature (27°C-29°C) growth and the other to 37°C growth. Using 27°C agglutinable antigen it was observed that the agglutination took

place slowly forming uniform sized small flakes. The sediment formed was compact, not easily dislodged but after vigorous shaking it dispersed as small granules in the clear fluid. The 37°C growth agglutinable antigen on the other hand agglutinated rapidly forming large flakes and producing a voluminous sediment which was easily dislodged on shaking giving rise to a homogenous suspension.

Cross agglutinations with immune sera prepared from 27°C growth of some typical Indian strain with the agglutinable antigen prepared from the other strains showed that the serum prepared from any one of the strains was capable of agglutinating all other strains practically to the same titre thus showing that there are no differences in the strains employed in our experiments. To correlate the results of the agglutination tests, agglutinin absorption tests were carried out with the antiplague sera, prepared from the 27°C growths of the representative strains of *P. pestis* stocked at this Institute. Out of the strains used four were Indian, one Egyptian, one South African and one from China. Each serum was absorbed with its own as well as other strains respectively and the absorbed serum put up with the homologous antigen. These experiments were repeated with a monovalent as well as a multivalent anti-plague serum prepared by immunising rabbits and horses with 37°C growth of the strains respectively. From these experiments it was observed that neither in the case of the room temperature (27°C 29°C) nor in the case of the incubator (37°C) growth, could any serological differences be detected between the various strains of *P. pestis* except in the case of a Chinese strain which may be a *pasteurella* type distinct from *P. pestis*. This strain also exhibited some cultural differences.

Agglutinin absorption tests with a serum obtained by immunising rabbits with 37°C as well as 27°C growths showed the existence of two types of antigen in *P. pestis*. It was observed that the 37°C growth was capable of absorbing all the agglutinins from the sera obtained by immunizing animals with the 37°C or 27°C growths, whereas the 27°C growth was only capable of removing the anti-bodies from a serum produced by immunizing animals with 27°C growths. In the case of 37°C growth antisera a residual portion was always left behind when the same was absorbed with the 27°C growth suspension and which subsequently reacted with the 37°C agglutinable antigen.

The incubator (37°C) growth when heated at 100°C for one hour behaved more or less like a room temperature growth. The antigenic differences were also shown by the characteristic of the specific types of agglutinations of the 37°C and the 27°C cultures.

It was also observed that by using the agglutination and agglutinin absorption tests, it was not possible to detect any serological differences between the virulent and the avirulent forms of *P. pestis* strain.

VI. A Serological Test—Diagnostic of Plague in Rodents.

(MENEZES.)

The studies regarding the diagnosis of plague infection in dead rodents by serological methods, was brought to completion during the year.

These studies have shown that plague infection in dead rodents can be definitely diagnosed serologically by putting up an emulsion of spleen or liver tissue against an adequate serum, the resulting reaction being made manifest by a heavy deposit in the tube consisting of agglutinated plague bacilli.

The serum to be utilised for the test must be one prepared against a 37°C growth of *P. pestis*.

Plague liver and spleens that have become putrid or kept for a month or more, either preserved in glycerine or desiccated, give the reaction just as well as fresh ones. The same organs from normal rodents or from those that have succumbed to any infection other than plague do not give the reaction. Considering that it is elicited within two hours of incubation, with material so scant as would be required to form 5/16 per cent. emulsion of the organs, it is obvious that the test would be an extremely useful one in coming to a rapid decision as to whether a dead rat sent for examination is plague infected or not. The fact that even putrid organs give a positive test still more enhances its value in as much as, in such cases, diagnosis by microscopic examination of smears or by culture or by passage is extremely difficult.

The liver or the spleen of all plague rats give a positive reaction, with the exception of those the smears of which show a sparse number of plague organisms. This is because the reaction is essentially on agglutination of the organisms present in the emulsion; hence tissues such as the muscle, brain, skin, testes, etc., smears of which ordinarily show no organisms, give no visible reaction.

The following are the salient points of the technique developed, full details of which along with a discussion of the rationale of the test will be given in a paper to be published shortly.

(1) It is preferable to work with a serum the titre of which is not less than 1 in 300.

(2) After taking smears on a few slides, 250 mgs. of the suspected spleen are emulsified in 10 cc. normal saline. This gives a 2½ per cent. emulsion and is made up to a final dilution of 1½ per cent.

(3) Three serum dilutions should be taken 1 in 10, 1—20, 1—40; the serum and emulsion being mixed in equal quantities (.3 or .5 cc. of each according to the size of agglutination tubes to be used).

(4) The test should be properly controlled with the following controls:—

(1) Normal saline control.

(2) Normal rat spleen emulsion control.

(3) Normal rabbit serum control.

(4) 1—4 dilution of a 5 weeks old broth culture of *Past pestis* grown at 37°C.

(5) The tubes are incubated in water bath at 40°C and readings taken after 2—3 hours.

VII. A Study of Some Virulent and Avirulent Strains of *Pasteurella Pestis*.

(WATS AND PUDÚVAL.)

Typical virulent and avirulent strains of *P. pestis* were studied for their colonial morphology, morphology of organisms, type of growth and the stability in broth. The preliminary work in this series was confined to the strains 120/5H virulent and avirulent. The strains were subcultured on superimposed rabbit blood agar and acid digest broth.

The virulent strains produced small, smooth, translucent glistening colonies with regular margins. The suspensions of the growth were salt stable. The organisms were ovoid and produced a homogeneous growth in broth. On subculture a majority of smooth colonies were reproduced at 27° C as well as 37° C. The avirulent strains produced a majority of large rough colonies with serrated or broken peripheries, the organisms being long and thin. The suspensions of the growth were salt sensitive and the type of growth in broth was granular. The study was extended to the primary cultures received from an epidemic area (Bettiah) in 1937-38. The cultures investigated were from the blood and buboes of septicæmic cases. In subculture three types of colonies, a smooth, a rough and an intermediate one were observed in these cultures. The smooth and the rough ones were identical with those obtained from the virulent and the avirulent strains respectively and the intermediate type which consisted of medium sized colonies with irregular margins and ruffled surface, sometimes showing a nipping in the centre or germination or at times appearing like a blob of pus. These colonies seemed to be a phase between the smooth and the rough forms, as the smear of the organisms showed both types of bacilli described above.

The presence of a soluble substance in the washing of the growth of the strains of *P. pestis* when grown at 37°C was tested. The strains were grown on acid digest agar, the growth suspended in adequate quantities of saline, heated at 55°C for 30 minutes and centrifugatized. The supernatants of some strains contained a specific soluble substance reacting with the anti-plague serum, the technique used being that of optimal proportions as described by Dean and Webb. It was observed that the presence or absence of the soluble substance has no relation to the virulence or non-virulence of the strains studied as avirulent strains e.g. Madagascar, Java, Egypt, 120/5H show its presence in varying proportions. It was however noted as a general rule that all virulent strains inevitably showed a high titre of the same though the titres varied from strain to strain. It was also observed that the presence or absence of the soluble substance was not dependent on the production of smooth or rough colonies by a strain, but as a general rule a strain producing majority of rough colonies was observed to be poor in the soluble substance content. The soluble substance contents of the strains seemed to show some correlation with their antigenic values.

VIII. The Metabolism of the Plague Bacillus.

(M. S. RAO.)

The study of the nutritional requirements of the organism has shown that three amino-acids (proline, phenylalanine and cystine) are necessary for growth in a chemically defined medium and that more complex growth factors such as thiamin, nicotinic acid etc., are not indispensable. The latter type of growth factors have acquired a considerable interest since the more recent elucidation of their role in metabolism. It has been found that some of them function as coenzymes in oxidation reductions and are universal constituents of the cellular metabolic apparatus. Their presence in the medium, when not essential for growth, should, therefore, confer a stimulatory effect on the growth and metabolism of the bacterial organism. This possibility in the case of the plague bacillus has been investigated experimentally by taking six compounds known to function as bacterial growth factors or as coenzymes of cellular oxidations, viz., (1) hæmatin, (2) cozymase or diphosphopyridine nucleotide, (3) nicotinic acid, (4) thiamin or aneurin, (5) alloxazine-adenine-dinucleotide and (6) B-alanine. In a chemically defined medium containing twelve amino-acids it was found that the first four compounds have considerable activity in shortening the lag period of growth of a well-washed inoculum, hæmatin being by far the most active. The four compounds hæmatin, cozymase, nicotinic acid and thiamin were also found in manometric experiments to stimulate the respiration (oxygen uptake) of cultures of the organism in nutrient broth. The specific effect of these compounds on a few of the more important oxidations catalysed by suspensions of the bacillus was also studied manometrically. It was found that only two reactions are stimulated; the oxidation of glucose is stimulated by cozymase, nicotinic acid and thiamin, and the oxidation of alanine by cozymase only. On the other hand the oxygen uptake of the suspension in the absence of added substrates is stimulated by hæmatin and cozymase only. Since these compounds occur also in the animal cell and therefore in the usual laboratory media prepared from meat and other biological sources, it is probable that they are important stimulatory factors in the growth and multiplication of the organism both in the host as well as in meat broth and similar media.

IX. The Effects of Temperature and Humidity upon the Growth of early Stages of the three Indian Rat-fleas.

(SHARIF.)

Further investigations on the effects of different constant temperatures and relative humidities on the rate of growth of larvae and pupæ of the three common Indian rat-fleas of the genus *Xenopsylla* were continued. About 20,000 recently hatched larvae of these species

were exposed to temperatures 13°, 17°, 22°, 27°, 32°, 35°, 36°, and 36·5°C. with relative humidities of 40, 45, 50, 60, 80, 90, 94·8, 97·5 and 100 per cent.

At 36·5°C. and humidities of 80 and 97·5 per cent larvae of *X. astia* bred successfully with a higher percentage of mortality at 80 per cent; but the larvae and pupae of *X. cheopis* failed to complete their development, and those of *X. brasiliensis* showed no signs of growth even at 36°C.

At 35°C. and 60 per cent humidity the larvae of *X. astia* died in the first instar, but at humidities 80 to 100 per cent they completed their larval and cocoon stages separately in 6-10 days. Over 90 per cent larvae of *X. brasiliensis* failed to grow at 60 per cent humidity. At this temperature and 80 per cent. humidity 13·5 to 30 per cent larvae of this species formed cocoons, but larvae in them failed to pupate, while at 90 to 100 per cent humidities 48·6 to 54 per cent larvae formed cocoons, and very few of them even managed to form pupae containing fully formed adults which failed to extricate themselves from the pupal skin. The desiccating influence of even 80 per cent humidity at this temperature is apparent. In the case of *X. cheopis* 62 to 73 per cent larvae reached the adult stage at 80 and 90 per cent humidities, but at 60 per cent humidity only 5·4 to 16·2 per cent larvae formed cocoons from which no adults emerged.

Though the durations of the larval and cocoon periods of all the three species were the shortest at 32°C. and optimum humidities with almost hundred per cent. success in rearing of adults, but the effect of high temperature retardation in the rate of increase in the velocity of growth at this temperature was prominent. The larvae of *X. astia* completed their larval and cocoon stages at 80 to 100 per cent humidities in 6-10 and 7-10 days respectively and at 60 per cent humidity in 10-15 and 7-10 days with over 50 per cent mortality in the larval stage. The larvae of this species did not grow at 50 per cent. humidity. The durations of the larval and cocoon periods of *X. brasiliensis* at 80 to 100 per cent humidities were 7-11 and 9-14 days, and at 60 per cent humidity, 9-14 and 8-12 days; but those of *X. cheopis* at 80 to 100 per cent humidities were 5-9 and 8-13 days, and at 60 per cent humidity 8-13 and 8-12 days. More than 90 per cent larvae of both these species died in the first larval instar at 50 per cent humidity and a very low percentage of them, however, grew, but none of them formed cocoons. Larvae of all the three species failed to grow at this temperature and 40 per cent humidity.

At 27°C. *X. astia* completed its larval and cocoon stages at 80 to 90 per cent humidities in 7-12 and 9-12 days and at 60 per cent humidity in 11-17 and 9-15 days. The larvae of this species showed no signs of growth at this temperature and 50 per cent or lower humidities. The durations of the larval and cocoon periods of *X. cheopis* at this temperature and 80 to 94·8 per cent humidities were 7-11 and 9-14

days, at 60 per cent humidity 8-14 and 10-14 days, and at 50 per cent humidity 12-20 and 10-16 days with almost hundred per cent success in rearing of adults; but those of *X. brasiliensis* were 8-12 and 10-16 days at 80 to 100 per cent humidities, 11-17 and 10-14 days at 60 per cent humidity and 18-23 and 12-15 days at 50 per cent humidity with 13-60 per cent success in breeding as opposed to almost cent per cent at higher humidities.

At 22°C. both the larval and cocoon periods were prolonged with almost hundred per cent success in breeding at humidities higher than 60 per cent. The larvae of *X. astia* completed their larval and cocoon stages at this temperature and 90-97.5 per cent. humidities in 11-17 and 13-24 days, at 80 per cent humidity in 13-19 and 13-18 days and at 60 per cent. humidity in 18-24 and 14-22 days. The larvae of this species did not grow at 50 per cent humidity. The durations of the larval and cocoon periods were in case of *X. brasiliensis* at 80 to 97.5 per cent humidities 11-17 and 14-25 days, at 60 per cent humidity 14-20 and 14-19 days, and in case of *X. cheopis* at 80 to 97.5 per cent humidities 10-17 and 13-22 days and at 60 per cent humidity 14-20 and 14-22 days. At this temperature and 50 per cent humidity larvae of both the species either failed to complete their larval life or a very low percentage of them reached the adult stage.

At 17°C. and humidities of 60 and 80 per cent the failure on part of defaecated larvae to spin cocoons meant sure death in the pupal stage. The durations of larval and cocoon periods in case of *X. cheopis* and *X. brasiliensis* at this temperature and humidities of 90 and 97.5 per cent were 23-36 and 29-45 days and in case of *X. astia* 28-41 and 27-41 days. The percentage of mortality was fairly high at 60 and 80 per cent humidities in all the three species.

At 13°C. larvae of *X. astia* would not grow at all at any humidity, though some of them lived for over a month at higher humidities. Larvae of both *X. cheopis* and *X. brasiliensis* died in the first instar at 60 per cent humidity, though some of them lived over two months without any growth. At this temperature and higher humidities of 80-100 per cent about 25 to 50 per cent larvae of both the species completed their larval life and died after four months; but a very low percentage of them, however, formed cocoons at 90 to 100 per cent humidities.

The effective range of humidity for larvae varies according to temperature, and this range of effective percentage of humidities is wider at 27°C. and gradually narrows down as the effective temperatures depart from this temperature which should not happen if a particular value of saturation deficiency is essential for the growth of the larvae. It is experimentally proved that the successful development of a flea larva depends on the water content of the food and that of the atmosphere. Effective humidity as a rule does not affect the rate of growth of the larva except when it is near the lower fatal limit, when the rate of growth is definitely retarded.

Failures and successes in rearing of adults at hundred per cent humidity led to the conclusion that excessive moisture by itself is not inimical to the growth of both the larvæ and the pupæ. Its injurious effect is due to the formation of a crust impermeable to air on the surface of the soil, the growth of fungi and putrefaction that sets in a soil rich in organic material. These factors prevent the supply of air necessary to larvæ and pupæ which die of suffocation. This brings us to the importance of the soil as a factor that should influence the increase and decrease of the flea population, and especially so, when we know that fleas are soil insects as all the early stages of fleas live in the soil and even a greater portion of the adult life is spent in the soil.

X. On the Male Genitalia of Siphonaptera.

(SHARIF.)

Previous studies on the male genitalia of *Ctenocephalides felis* subsp. *orientis* (Jordan) were extended so as to include the account of these organs in some of the species of *Xenopsylla* and other genera. The ontogenetic studies on the male genitalia of the three Indian species of *Xenopsylla* have led to the conclusion that the so-called ninth abdominal sternum is a composite structure, consisting of a vertical portion which is a sclerotized portion of the ninth abdominal sternum and a horizontal portion which is formed by the fusion of the subgenital plates or harpagones. The phallic apodemes are the parameres. Though they are quite distinct in the prepupal stage but they become incorporated in a complicated phallic complex of the pupa, which is a prominent structure at the posterior end of the ninth abdominal segment of the pupæ of *Nosopsyllus fasciatus* (Hose) *Ceratophyllus gallinae* (Schrank) and *Leptopsylla segnis* (Schönherr), but becomes completely withdrawn in the ninth abdominal segment of the pupæ of species of *Xenopsylla* and *Ctenocephalides*. The male pupæ of all the seven species of fleas examined can easily be distinguished from the female pupæ by the examination of their terminal abdominal segments.

XI. Typhoid Fever in the City of Bombay.

(WAGLE.)

As the Government had sanctioned the continuation of Enquiry, this Institute continued to give free diagnostic aid to private medical practitioners in suspected typhoid cases and carried on the work of detection of carriers.

Diagnosis of cases.—The procedure adopted was the same as last year. On a call from a medical practitioner, an officer of the Institute visited the patient and obtained material for blood culture and the Widal test. In cases such as children where there was difficulty in getting blood,

examination of faeces and urine was suggested. Thus from 1st January to 31st December 1939, the Institute received 575 calls from the medical practitioners of the City and suburbs.

(i) *Blood culture*.—This year the blood culture was made in 20 cc. sterile OX-bile instead of in 100 cc. glucose broth. 10 cc. of blood was drawn aseptically from a vein, if the patient was an adult and 5 cc. if the patient was a child. Half the quantity drawn was used for making a culture in OX-bile and the remaining half was put aside for obtaining serum for the Widal test. The clot remaining after the separation of serum was used for making another culture in OX-bile. Both the OX-bile cultures were examined for growth after incubation for two days. Those that gave negative results after two days were re-examined after 5 days.

(ii) *Widal test*.—Dreyer's technique was adopted for the Widal test. As before a titre of 1 in 250 with Bact. typhosum 'O' suspension, and a titre of 1 in 125 with Bact. typhosum 'H' suspension and of 1 in 25 with Bact. paratyphosum A. H. suspension were taken as of diagnostic significance.

(iii) *Culture of faeces and urine*.—Wilson and Blair's bismuth sulphite agar was used for isolation of organisms from faeces and urine of patients, as well as of carriers.

Thus out of 575 cases examined by this Institute, 256 were diagnosed as those of enteric infection. 223 of these were cases of typhoid fever, and 33 of paratyphoid A fever. In 127 cases of typhoid fever Bact. typhosum was isolated from blood, while in the remaining the diagnosis was based on positive Widal test. Of 33 paratyphoid A cases, 21 were diagnosed by positive blood culture, the rest by the Widal test. Again no case of paratyphoid B fever was detected in the City.

The following table gives the distribution of these 256 cases.

Ward ..	A	B	C	D	E	F	G	Suburbs.
Number of cases.	3	14	23	36	2	69	55	54

Again as last year the Wards F, G and D contributed to more than half the number of cases, i.e., 160 cases. Amongst the suburbs, Bandra and Santa Cruz were the worst to suffer, and together had 30 cases.

Carriers.—This year in order to facilitate the work of detection of carriers, it was decided to examine as many persons as possible from amongst those who had suffered from typhoid fever during the previous

year. With this idea in view a circular was sent to the medical practitioners requesting them to try and send the samples of faeces and urine of their old patients as well as of those who had recently suffered so that it would be possible to see if any of their old patients had become carriers. Unfortunately the response to this appeal was very poor.

The other group of persons examined were those who for some reason or other were suspected to be carriers, though they were actually not known to have suffered from typhoid fever. Thus in the case of two dairies, where milk supply was not above suspicion, their personnel was examined for carrier state.

The procedure adopted for examination was as follows. In cases whose blood samples were available, the blood was examined for the presence of Vi antibody ; if Vi antibody was found to be present then the faeces and urine of that person were examined ; if it was absent, then examination of faeces and urine was not done. In others where blood was not available for 'preliminary' examination urine and faeces were examined. In most cases as repeated samples of urine and faeces were not available, we had to be content with a single examination.

In all 115 persons were examined for carrier state, and only one carrier was detected amongst them. The faeces of this suspect were sent by the Arthur Road Hospital and *Bact. typhosum* was isolated from the sample.

XII. Vi Agglutination in the Diagnosis of Typhoid Fever.

(WAGLE.)

The value of Vi agglutination in the detection of typhoid carriers was first shown by Felix and later confirmed by various workers. Bhatnagar later showed its importance in the diagnosis of acute typhoid infections in inoculated persons, in whom O and H agglutinations are not of much value. He found it to be extremely useful not only in diagnosing typhoid infections in inoculated individuals but also in the uninoculated, provided repeated examinations of blood were done. But repeated examinations of blood, though possible among men under military discipline and in hospitals, cannot be easily done in private practice and reliance is often to be placed on a single serological examination. Of what value Vi agglutination would be under these conditions was not known and since this Institute was giving free laboratory aid to the medical practitioners in the diagnosis of typhoid infections, it was decided to take this opportunity and examine the sera collected from these patients, for the presence of Vi antibody. The sera of only those patients from whom the organism was isolated were tested for the presence of this antibody. Along with this O and H agglutination was also done as a routine. Preserved agglutinable "Vi I" suspensions obtained from Entertic Laboratory, Kasauli were used for the Vi agglutination. Standardised

formolised broth cultures prepared after Dreyer's technique of 'O' 901 and 'H' 901 strains of *Bact. typhosum* were used for O and H agglutination. In all, sera from 92 patients were examined, of which 83 were uninoculated individuals and 9 inoculated ones. The results of Vi agglutination along with those of O and H agglutination obtained in 83 uninoculated patients are summarised in the following table :—

Period of disease when blood was collected.	Total No. of cases examined	Total No. of cases diagnosed by O, H and Vi agglutination.	No. of cases diagnosed by O & H agglutination.	No. of cases diagnosed by Vi agglutination.	No. of cases diagnosed by Vi agglutination alone.	No. of cases diagnosed by O & H agglutination alone.
1st week	17	16	13	11	3	5
2nd week	50	42	36	31	6	11
3rd week and later ..	16	16	16	14	0	2
Total ..	83	74	65	56	9	18

It would be seen that the use of Vi agglutination enabled us to diagnose 9 cases which would otherwise have been missed by the routine O and H agglutination. But it also gave negative result in 18 cases which were diagnosed by O and H agglutination. It would therefore appear that Vi agglutination though certainly of great value as an accessory to the routine O and H agglutination cannot entirely replace it especially when repeated examinations of blood are not possible. In the inoculated patients, however, it is definitely more useful because in them the routine O and H agglutination has very little significance. Out of the 9 inoculated patients in this series 7 showed the presence of Vi antibody.

XIII. Potency of Some T. A. B. Vaccines on the Market.

(WAGLE.)

During the typhoid epidemic it was found that besides the vaccine issued by this Institute a number of commercial vaccines were used in the City for prophylactic purposes. As a number of medical practitioners made inquiries in this Institute as regards their protective power samples of those available locally were obtained and tested for their potency in laboratory inbred white mice. The mice were first immunised by giving them different vaccines and subsequently the protection developing in them was assessed by infecting them with a highly virulent typhoid strain. Along with the commercial vaccines,

a sample of the Institute vaccine was also tested for comparative purposes. The following table gives a summary of the number of experiments made with these vaccines :—

Vaccine used for immunization.	Number of experiments done.	Total number of animals immunised in the experiments.	Number of animals dead after giving the infective dose.	Percentage mortality.	Controls.
No. 1 Haffkine Institute Vaccine ..	4	23	2	8.70	Controls showed 100 % mortality in all experiments.
No. 2 Commercial Vaccine ..	3	17	11	64.70	
No. 3 do. ..	3	17	8	47.05	
No. 4 do. ..	3	18	11	61.11	

It will be seen that No. 1 vaccine shows the highest degree of protection. Next in order comes No. 3 vaccine. The different batches of vaccines were also seen to vary in their protective power. Thus one batch of a commercial vaccine gave good protection to mice while another batch gave hardly any.

XIV. Isolation of the Decomposition Product of Plasmoquine.

(DIKSHIT AND NANDI.)

As indicated in the last year's report the nature of destruction of plasmoquine by the body tissues was investigated and further evidence was brought forward to show that the agents responsible for destruction of this drug were probably enzymes. The organs which were chiefly responsible for this destruction were found to be in order of their activity as follows:—Brain, liver, spleen, testis and kidney. Saline extracts of these organs were also found to exert this action. Hydrochloric acid and cyanides were found to inhibit the plasmoquine destroying property of organ extracts and the optimum temperature for destruction was observed to be in the neighbourhood of 40° C.

Successful attempts were made to isolate the product of decomposition of plasmoquine. For this purpose the liver was perfused with Locke's solution by a specially devised apparatus and thus kept artificially alive for more than five hours. Plasmoquine solution was passed through such isolated organs and the substance into which plasmoquine was converted was isolated by chemical extraction.

The method of chemical extraction involved precipitation of proteins by trichloroacetic acid, removal of the undecomposed plasmoquine by ether and recovery of the decomposed product of plasmoquine as its phosphotungstate.

XV. Pharmacological Action of the Decomposition Product of Plasmoquine.

(DIKSHIT.)

The pharmacological action of the decomposition product of plasmoquine was studied. Toxicity tests showed that it is considerably less toxic than plasmoquine itself. Its action on the cardiovascular system is less marked than that of plasmoquine and even when given in large doses it does not produce cardiac irregularities which is a constant feature after moderate doses of plasmoquine. The decomposition product increases the movements of the gut and its tone which action is exactly opposite that of plasmoquine. The product however resembles plasmoquine in its action of methaemoglobin formation.

XVI. Mode of Action of Atebrin in Monkey Malaria.

(DIKSHIT.)

In order to see the possibility of a direct lethal action on the malarial parasite (*Plasmodium Knowlesi*) *in vitro* experiments with the defibrinated blood of infected monkeys were made and it was found that 1:200,000 concentration of Atebrin acting at room temperature for 24 hours is unable to kill the parasites. A 1:100,000 concentration acting for 24 hours usually kills them but this concentration acting for 6 hours at body temperature does not affect the parasites. It is not possible for such high concentration of 1:100,000 of Atebrin to be present in the circulating blood except perhaps for a few minutes and thus a direct lethal action of the drug on the parasites in the circulating blood is not probable.

Attempts were then made to see if the reticuloendothelial system played any role in the curative action of Atebrin in monkey malaria. For this purpose the reticuloendothelial cells were blocked by large intravenous doses of India ink in splenectomized monkeys and it was found that in such animals the usual curative dose of Atebrin failed to bring about a complete cure. Further work on these lines is in progress.

XVII. Immunity in Monkey Malaria.

(DIKSHIT.)

The exact nature of immunity in malaria is not yet known and some recent work has suggested that the nature of immunity may be humoral. To study this problem a technique of cross circulation was evolved which permits of the blood of one monkey being replaced by that of one or more other monkeys. By replacing the blood of an immune monkey with that of non-immune monkey and *vice versa* evidence has been collected which goes to show that immunity in monkey malaria does not reside in the blood but is present in the body tissues. This interesting problem is being pursued further.

XVIII. Respiratory Metabolism of Normal Tissues in the presence of Plasmoquine.

(NANDI.)

The effect of plasmoquine at various concentration on the oxygen metabolism of normal tissues and blood was studied with the Barcroft differential manometers. It was observed that at certain concentrations plasmoquine stimulated considerably the oxygen uptake of normal guinea-pig spleen, liver, brain, defibrinated blood, and cell-free liver extract. Generally three different concentrations of plasmoquine had been investigated in the case of tissues, e.g., 1 in 6,000, 1 in 15,000 and 1 in 150,000. A fourth concentration, e.g., 1 in 300,000 had in addition been taken in the case of blood.

Although there was no exact quantitative relationship between the co-efficient of stimulation and the concentration of plasmoquine, generally the stimulation was highest at 1 in 6,000 and the lowest at 1 in 150,000 concentration in the case of tissues.

The stimulation of oxygen uptake was most prominent in the case of blood. At 1 in 6,000 concentration of plasmoquine the co-efficient of respiratory stimulation was in one case 1436.3 per cent.; the most remarkable feature being that even at a concentration of 1 in 300,000 of plasmoquine, which is usually the level reached by the drug in blood when given in therapeutic doses, the co-efficient of stimulation was 41 per cent.

It was observed that the stimulatory effect of plasmoquine in tissues, blood and cell-free liver extract was abolished almost to the same extent as normal respiration by the action of heat or cyanide.

With a view to establish a quantitative relationship between the percentage co-efficient of respiratory stimulation and the destruction of plasmoquine, it was observed that normal tissues which destroyed the

least amount of plasmoquine exhibited the highest respiratory stimulation and *vice versa*. It was, however, established that the phenomenon of stimulation of oxygen uptake by tissues, blood and cell-free liver extract in the presence of plasmoquine was definitely an enzymatic (thermo-labile) reaction, whilst the decomposition of plasmoquine itself was mainly through non-enzymatic (thermo-stable) systems present in the tissues.

XIX. Chemotherapy of Bacterial Infections.

(GANAPATHI.)

The striking results obtained with sulphanilamide and 2-sulphanilamidopyridine (M and B 693) in the treatment of some bacterial infections has given us hope of successfully tackling other bacterial diseases also. The object of the present studies is to discover derivatives of sulphanilamide which may prove either superior to the two above-mentioned drugs in clinical practice or effective in diseases in which they fail.

Since the past experience clearly indicates that substitutions at the amino radical of sulphanilamide greatly decreases the activity and suppress the polyvalent action of the mother compound, sulphanilamide derivatives with different substituents in the sulphonamide part of the molecule have been synthesized. N-sulphanilylglycine and N-sulphanilyltaurine were devoid of activity while N-sulphanilyl-phenylalanine was less active than sulphanilamide. The three isomeric nitro derivatives of 4-aminobenzenesulphonanilide were more active than sulphanilamide and more toxic. Since it appears that compounds more polyvalent in action than sulphanilamide are obtainable by the substitution of heterocyclic derivatives at the sulphonamide radical, many types of heterocyclic derivatives were tried. Two dyes were prepared by diazotising 2-sulphanilamidopyridine and coupling it with metaphenylenediamine and 4-aminothiouracil. The former stained the tissues too much while the latter was far inferior to the parent compound. The compound 2-(sulphanilamidobenzene sulphonamido) pyridine was of far inferior activity. 6-sulphanilamidoquinoline was extremely toxic while 2-Sulphonamidobenzene aminoquinoline 3-carboxylic acid, though comparatively less toxic, possessed very little activity. 2:8-Sulphanilamidoacridine and a series of 9-substituted acridine derivatives by condensing 9-chloroacridine with sulphanilamide, 2-sulphanilamidopyridine, 4-sulphanilamidobenzene-sulphonanilide, etc., have been tested. Some of them show considerable activity in experimental streptococcal infections. Of all the compounds synthesized, 2-sulphanilamidothiazol was found to exhibit a remarkable protective effect in experimental streptococcal and pneumococcal infections in mice. Similar derivatives of the above thiazol compound are being synthesized and studied.

XX. Effect of Sulphanilamide Derivatives in Streptococcal and Pneumococcal Infections.

(R. S. RAO AND K. GANAPATHI.)

New sulphanilamide derivatives which were synthesised in this Institute with a view to discovering a drug effective in plague, were also tested in haemolytic streptococcal and pneumococcal (Type I) infection of mice.

The mice used in these experiments were of the Haffkine Institute inbred strain. The strains of organisms employed in the tests were the "Richards" strain of haemolytic streptococcus and a pneumococcus Type I strain. For purposes of experiment 17 hours old cultures of the organisms in serum broth were used. The mice were infected intraperitoneally with a dose which killed them within 48 hours. The drugs were made into an emulsion in gum acacia such that 0.5 c.c. of the emulsion contained the required dose. This quantity of the emulsion was introduced into the stomach of the mouse by means of a pipette. So far twenty new derivatives mainly of the typical heterocyclic group such as pyridine, quinoline, acridine and thiazole have been tested.

Of these one derivative namely sulphathiazole proved to be distinctly superior to sulphanilamide in haemolytic streptococcal infection and as good as sulphapyridine in pneumococcal (Type I) infection.

10 mg. of this drug administered to mice infected with "Richards" strain of haemolytic streptococcus soon after infection and repeated 6, 24, 48, 72 hours later protected 70 per cent. of the mice, while with the same dosage sulphanilamide protected only 30 per cent.

In pneumococcal (Type I) infection both sulphathiazole and sulphapyridine 22½ mg. administered twice a day at 12 hourly intervals for six days protected all the animals.

It was also found that this drug is much more effective when given in 2 or 3 divided doses than when the same amount is given as a single dose.

XXI. Study of Blood Urea Clearance in Normal Indians.

(S. K. GOKHALE.)

From the routine determinations of blood urea clearance test carried out in this Institute, it was observed that invariably low values were obtained in comparison to the average normal blood urea clearance value given by Möller, McIntosh and Van Slyke (Jour. Clin. Invest. 1929, 6, 427), based on a study of normal American subjects.

Work done in this Institute had shown that in the case of normal Indians, while their blood urea content was within the same limits as that of American normal subjects, the urinary urea content, on the other hand, was about half of that of American subjects. In view of this fact, it was thought necessary to investigate whether the blood urea clearance value for average American adult will also hold good for the average Indian adult.

Blood urea clearance values of about one hundred normal Indian male subjects have been examined. The same technique as described by Möller McIntosh and Van Slyke has been followed. The average values obtained are 44 c.c. for maximum clearance and 34 c.c. for standard clearance. The corresponding averages for Americans were 75 c.c. and 54 c.c. respectively.

This shows that the use of American averages to evaluate the renal function in the case of Indians would lead to erroneous conclusions. The study is being continued further.

XXII. The Destruction of Airborne Bacteria.

(WATS.)

In a bacteriological laboratory, in the media and vaccine rooms, or in rooms where tissue culture work is being carried out, the sterilization of the air is of decided advantage. The fungus infections during the monsoons in India are so common that any method ensuring even a partial success would be welcome. Pulvertaft and others (1939) have carried out an extensive series of tests by atomization of bactericidal solutions with "Phantomyst" an electrically operated apparatus capable of introducing the liquid antiseptic into the atmosphere in the form of a dry and penetrating mist called "Acrosol".

TECHNIQUE.

(a) One of the laboratory rooms of capacity 5,650 cubic feet was used for the experiments. The ceiling fan of this room was kept working during the experiments.

(b) A solution obtained by mixing equal parts of glycerine and saturated aqueous solution of resorcinol was used.

(c) Cultures of *B. prodigiosus*, *staphylococcus albus*, *streptococcus faecalis* and *B. coli* were used as test organisms. Watery emulsion of the growth was sprayed in the test room by means of De Vilbiss hand power spray outfit.

(d) Two types of power driven machines were used. (1) The Phantomyst Model A1 and (2) the AMCO No. 4 Atomizer with power driven air compressor for details of these machines, refer to the Journal of the Malaria Institute of India 3, 1, June 1940, page 129.

DETAILS OF EXPERIMENTS.

Control experiment.—Suspensions of *B. prodigiosus* (200,000 million organisms) were uniformly sprayed by De Vilbiss hand power pump. Petri-dishes containing nutrient agar were exposed for 10 minutes at three different parts of the floor of the room half to one hour after the introduction of the organisms. The exposed plates were incubated at 37°C for 48 hours and colonies counted. It was found that innumerable colonies were present on the plates exposed after 30 minutes and more than 1,000 colonies on those exposed after 60 minutes.

A similar experiment was carried out without working the ceiling fan showed innumerable colonies on immediate exposure and only about 10 colonies after 15 minutes, hence importance of keeping the ceiling fan working.

TEST EXPERIMENT.

Phantomyst.—Similar procedure was adopted for the test experiments, except that the Phantomyst Machine containing the resorcinol solution was started immediately after dispersion of the organisms. To test the effect of the various quantities of the disinfectant the machine was run for 15, 30 and 60 minutes respectively in separate series of experiments. At the end of quarter, half and 1 hour 5, 8 and 12 c.c. of the solution were blown on an average. The colonies, after running the machine for quarter hour, were numerous, while two to three colonies were always found after half and one hour's running. From these experiments it was evident that a minimum quantity of 8 c.c. of the resorcinol solution was essential to sterilize the atmosphere of the room (5,500 cubic feet).

Atomizer.—The above experiment was repeated by replacing the Phantomyst with an air compressor and AMCO No. 4 Atomizer which is used for humidifying atmosphere in cotton mills in Bombay. The aqueous solution of *B. prodigiosus* was dispersed as before and then the disinfectant was atomized by means of the above apparatus at a pressure of 30 lbs. various amounts of the disinfectant were used to find the minimum quantity and time necessary for complete sterilization. In this case of the operator has to direct the spray into all parts of the test chamber.

The other procedures were similar to the experiments described above. It was found that 12 c.c. of the glycerine resorcinol solution would give similar results to the Phantomyst experiment, i.e., an average of 2 or 3 colonies per plate after quarter as well as half an hour. For complete sterilization of the room at least 25 c.c. of the solution were necessary. Other organisms were now tried with the same result. A few experiments were also tried with 5 per cent. Chloramine T. solution using *B. prodigiosus* suspensions for the test and found to give similar results both in the case of the Phantomyst as well as the Atomizer.

EFFECT ON CULTURE MEDIA.

In the case of Phantomyst superimposed 5 per cent. rabbit blood agar slopes were directly held for about 10 minutes over the outlet so that the mist of glycerine resorcinol solution entered the test-tube. These tubes as well as the unexposed tubes were inoculated with *Past pestis*. After inoculating at 37°C and room temperature, the number of colonies were found almost to be the same. In the case of the atomizer the test tubes containing the blood agar media were exposed in the room previously sprayed with the glycerine resorcinol solution and cultured. No inhibition of growth was observed.

Practical application.—This method of sterilizing the air was introduced into the plague vaccine sowing room and the antirabic department. Though it has been in practice for about a month only the results are very encouraging.

XXIII. The Choice of Mechanical Sprayers for Mosquitocides Sprayed for Anti-malarial Purposes.

(WATS AND BHARUCHA.)

Although the control of malaria by killing adult mosquitoes in dwellings has become a recognised procedure as supplementing antilarval measures or superseding these where conditions are unfavourable for the latter, it is surprising that no satisfactory sprayer has been recommended for wholesale measures. It was with the object of studying the relative efficiency of the various types of apparatus that could be used for spraying mosquitocides that this investigation was undertaken.

The unsatisfactory results obtained by using hand sprayers indicated :—

- (a) the use of power driven air compressor outfits.
- (b) the maintenance of exact relationship of the air and liquid orifices in the nozzle.
- (c) the provision of a nozzle that does not begin to spray until sufficient air pressure is obtained to produce a good spray, and ceases to spray before the air pressure drops to a point where good spraying is impossible.

Six types of sprayers were tried. The experiments were carried out in an ordinary laboratory room with well-fitting doors and windows. Mosquitoes were imprisoned in mosquito-netting bags in different parts and at different levels of the room. Mixed catches of mosquitoes were used, the majority being *Armigeres obturans*, a hardy culecine mosquito. Measured quantities of mosquitocide of known potency

were blown off from the various sprayers and the room kept closed for half an hour. The bags were then removed to fresh air and the results recorded the following morning.

Hand sprayers were found to be of limited use. The De Vilbiss Hand power spray outfit was found to be satisfactory but of limited use. Spray painting guns with power driven air compressors gave very satisfactory results but as the air compressor, the spray gun, the air cap, the fluid tip are all interdependent, careful selection was necessary. The AMCO atomizer No. 4 was found to be the most satisfactory and as the nozzle does not work below a stipulated pressure there is no dripping.

The Diffusor in which the insecticide is atomized by steam and the Phantomyst which converts the mosquitocide into a cloud were found to be unsatisfactory. *In Press.*

XXIV. Intravenous Injection of Oxygen under Normal Atmospheric Pressure.

(INDERJIT SINGH AND SHAH.)

Experiments carried out during the year showed that in human beings about 10-20 cc. of oxygen can be injected into veins directly without any untoward effect under normal atmospheric pressure. It is expected that if the atmospheric pressure is increased then considerable more quantities of oxygen could be administered.

XXV. The Effect of Temperature on the Mechanical Response and the Viscosity and Oxygen Consumption of Unstriated Muscle.

(INDERJIT SINGH AND M. S. RAO.)

The effect of temperature on unstriated muscle has been described by several workers, but since it was found that the two excitabilities in unstriated muscle are affected differently by various agencies, it was deemed expedient to determine the effect of temperature, as this also does not affect them identically. As temperature may affect the viscosity of the muscle, certain experiments were performed to elucidate this probable action, and as viscosity has been considered to play an important part in enabling unstriated muscle to maintain tonic contraction without expenditure of energy, the oxygen consumption of muscles under various experimental conditions was recorded.

XXVI. The Effect of the Interaction of Ions, Drugs and Electrical Stimulation as indicated by the Contraction of Mammalian Unstriated Muscle.

(INDERJIT SINGH.)

At the temperature of 37° C the unstriated muscle of cold blooded animals becomes practically inexcitable. It was found that one of the factors causing this inexcitability in the muscle of cold blooded animals was rapidity of adaptation.

XXVII. Tonus and the Process of Inhibition in Unstriated Muscle.

(INDERJIT SINGH.)

In the guineapig uterus, most agencies that cause contraction in other plain muscles produce inhibition. This is due to the fact that the guineapig uterus exhibits much tone. This tone is produced by chloride of the saline, which antagonises the action of cations, which are necessary for contraction.

XXVIII. The Effect of Ammonium and some other factors on the Potassium Content of Unstriated Muscle and its relation to the Contraction Produced on Withdrawal of Chemical Substances from around the Muscle.

(INDERJIT SINGH AND S. K. GOKHALE.)

There are two kinds of withdrawal contractions, one produced by ammonium and having the properties of the A C contraction and the other by thiocyanate having the properties of the potassium contraction. It has been presumed that the former contraction is produced by ammonium ions within the muscle fibres, and the latter by ions without the fibres. Chemical analyses support these conclusions, as it has been found that the muscle is more permeable to ammonium chloride than to sodium chloride, and more to the latter than to sodium thiocyanate.

XXIX. Elution of Fixed Rabies Virus.

(R. S. RAO AND R. K. GOKHALE.)

One of the well-known methods of isolation in pure form of viruses is a preliminary absorption and subsequent elution. Attempts were made by the application of this technique to isolate fixed rabies virus

in pure form from nerve material. The procedure employed was as follows:—

To one volume of aluminium hydroxide (2·24 per cent. of Al_2O_3) is added an equal volume of Walpole's acetate buffer solution at the desired pH. To this mixture is again added an equal volume of a 10 per cent. suspension of rabbit brain fixed virus in saline. This final mixture is kept in cold for four hours. This is next centrifuged for 10 minutes. The supernatant fluid is discarded and the sediment is washed by suspending it in distilled water and again centrifuged. The sediment is finally suspended in KH_2PO_4 buffer solution at pH 8 and kept in cold for over three hours. The suspension is then centrifuged for 10 minutes and the clear sterile supernatant is tested on rabbits by subdural inoculation for the presence of the virus. Experiments conducted at pH of 3·6, 4, 4·4, and 5 for absorption were not successful. Further experiments are being made adopting higher ranges of pH for absorption.

XXX. Inquiry into the Nutritive Value of Sweet Toddy.

(SEVERAL WORKERS.)

The value of sweet toddy was investigated from its nutritional point of view and as the investigations revealed that sweet toddy is a drink of high nutritive value, experiments were undertaken to see if toddy could be preserved for some time without allowing it to ferment and without deterioration of its important nutritive constituents.

Fresh toddy tapped from cocoanut, date or brab palms is a refreshing sweet drink and its freshness can be preserved for several days if it is collected in clean nonporous jars, chilled immediately on the spot and preserved in the cold at a temperature of about 4°C . Chemical analysis of a large number of samples was made and it was found that cocoanut toddy has the following approximate constituents of some nutritional value, viz. :—

Proteins	.. 0·1 to 0·2 per cent.
Non-protein nitrogen	.. 25 mg. per cent.
Total Nitrogen	.. 50 mg. per cent.
Fe	.. 0·5 mg. per cent.
Ca	.. 0·06 per cent.

The most important constituents however were found to be the sugars and the vitamins. The total carbohydrates in a fresh sample of sweet cocoanut toddy was found to be about 8 to 10 per cent. Levulose formed one of the chief ingredients of the carbohydrates and was present in fresh samples in quantities varying between 3 and 5 per cent. Levulose is a sugar of considerable medicinal value and its presence in the toddy in high

proportion is significant. The other important constituents were the vitamins. We found that vitamin C was present in fairly large amounts, the average percentage being approximately 10 mg. per cent. It was noted that the vitamin C content varied considerably in samples of toddy collected from different localities and also from different trees in the same locality. The other important vitamin that was estimated was the vitamin B₁. There is always some yeast present in fresh toddy and this yeast was evidently the source of vitamin B₁. Our results go to show that there are in a fresh sample of toddy approximately 1 to 3 International Units of vitamin B₁ per 100 cc. One would naturally expect that if the toddy is allowed to ferment the amount of yeast in it will increase considerably and also the amount of vitamin B₁ proportionate to the increase in the yeast cells. In some of our experiments, however, under controlled fermentation even a ten-fold increase in the number of yeast cells raised only slightly the vitamin B₁ content of the toddy. Thus a sample which had a B₁ content of 2.5 I.U. per 100 cc. before fermentation, had just 3.0 I.U. after 48 hours fermentation. This apparent discrepancy is now under investigation.

Bacteriological study of sweet toddy.—A fresh sample of sweet toddy collected in sterilized glass pots and chilled immediately on the spot was found to contain a variety of organisms. These could be classified into four groups viz. :—

(1) A gram negative, nonmotile, coliform bacillus which ferments lactose with gas.

(2) An organism similar to above but motile.

(3) A gram positive, diplococco-bacillus, which is pleomorphic and non-lactose fermenter and does not produce gas in sugars which are fermented.

(4) Yeast.

All the above types of organisms were found to be destroyed by a 0.1 per cent. concentration of sodium benzoate except the gram positive diplococcobacillus.

Preservation of sweet toddy.—Toddy collected in the usual way in earthen pots which are rarely cleaned, has a peculiar mockish taste. This taste can be eliminated if a non-porous clean pot is used for collection. All our experimental samples were collected in the sterilized glass pots and the pots were cleaned and sterilized everytime before use. The collections were made early in the morning, the juice put in sterilized glass containers and brought to the Institute preserved in ice. The juice was then filtered through several layers of sterilized gauze, put in suitable flasks and pasteurized at 80° C for 35 minutes. It was immediately cooled down to 5° C. Sufficient quantity of sodium benzoate was added to give a concentration of 0.1 per cent. The juice was then poured in sterilized crown cap bottles, charged with CO₂ at 40 lbs. pressure and the crown

caps applied with a hand-cap machine. The bottles were then put on an ordinary table without taking any further precautions to preserve them. They were then opened at definite intervals and the preserved toddy subjected to a detailed chemical and bacteriological analyses. It was found that this method of preservation prevented yeast fermentation and the important food constituents like the sugars and vitamin remained as they were for a period exceeding 8 weeks. Preservation in cold storage in addition to the details given above preserved the toddy for a much longer period.

LIST OF PAPERS PUBLISHED FROM THE HAFFKINE INSTITUTE DURING THE YEAR 1939.

1. *Dikshit, B. B.*—"Plasmoquine in Pregnancy, with special reference to the action on the Foetus." *Journal, Malaria Institute of India*, Vol. 2, No. 4, December 1939, pp. 387-393.
2. *Ganapathi, K.*—"Action of Sulphanilamide Derivatives in Streptococcal and Pneumococcal Infections in Mice." *In Press*.
3. *Ganapathi, K.*—"The Configuration of the C₃ hydroxyl group in Digitonin Precipitable Steroids." *Current Science*, 1939, 8, 360.
4. *Ganapathi, K.*—"The Configuration of the C₂ and C₃ hydroxyl groups in Gitogenin and Digitogenin." *Current Science*, 1940, 9, 18.
5. *Gokhale, S. K.*—"Non-Protein Nitrogenous Constituents of Blood": A study based on the examination of 126 normal Indian Men. *Indian Journal of Medical Research*, Vol. 26, No. 3, January 1939, pp. 675-697.
6. *Rao M. Sadashiva.*—"The Nutritional Requirements of the Plague Bacillus." *Indian Journal of Medical Research*, Vol. 27, No. 1, July 1939, pp. 75-89.
7. *Rao M. Sadashiva.*—"Oxidations effected by the Plague Bacillus." *In Press*.
8. *Rao M. Sadashiva and Singh, Inderjit.*—"The Effect of Temperature on the Mechanical Response and the Viscosity and Oxygen consumption of Unstriated Muscle." *In Press*.
9. *Row, R.*—"Some experimental observations on Human and Rat Leprosy and their significance in the pathogenesis and treatment of the disease." *Trans. Roy. Soc. Trop. Med. Hyg.*, Vol. 32, No. 4, January 28, 1939, pp. 497-504.
10. *Singh, Inderjit.*—"A Comparative Study of the Effect of the Interaction of Ions, Drugs, and Electrical Stimulation as indicated by Contraction of Unstriated Muscles." *Journal of Physiology*, Vol. 96, No. 4, pp. 367-384.

11. *Sokhey, S. S.*—"Experimental Studies in Plague. Introduction, Part I." *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 313-319.

12. *Sokhey, S. S.*—"Experimental Studies in Plague, Part II": The Solid Medium of Choice, and the Optimal Temperature of Incubation for the Growth of the Plague Bacillus. *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 321-329.

13. *Sokhey, S. S.*—"Experimental Studies in Plague, Part III": A Method for Determining the Number of Viable Plague Organisms in Broth Cultures. *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 331-340.

14. *Sokhey, S. S.*—"Experimental Studies in Plague, Part IV": Experimental Animal of Choice for Plague Work. *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 341-354.

15. *Sokhey, S. S.*—"Experimental Studies in Plague, Part V": A Method for Measuring the Virulence of Plague Cultures. *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 355-361.

16. *Sokhey, S. S.*—"Experimental Studies in Plague, Part VI": A Method for Maintaining the Virulence of *Pasteurella Pestis*. *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 363-371.

17. *Sokhey, S. S., Chitre, G. D., and Gokhale, S. K.*—"The relative Value of some Proprietary Cyanide preparations for the Extermination of Rats and Fleas as a Plague Preventive Measure." *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 389-407.

18. *Sokhey, S. S., and Malandkar, M. A.*—"Basal Metabolism of Indians." : A Study based on the Examination of Sixty Normal Indian Men. *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 501-529.

19. *Wats, R. C., Wagle, P. M., and Puduval, T. K.*—"A Serological Study of some Strains of *Pasteurella Pestis*." *Indian Journal of Medical Research*, Vol. 27, No. 2, October 1939, pp. 373-388.

20. *Wats, R. C., and Bharucha, K. H.*—"The Choice of Mechanical Sprayers for Mosquitocides sprayed for Anti-malarial Purposes." *In Press*.

21. *Wats, R. C., and Kamat, G. K.*—"The Destruction of Airborne Bacteria." *In Press*.

22. *Wats, R. C., and Puduval, T. K.*—"A Study of some Virulent and Avirulent Strains of *Pasteurella Pestis*." *In Press*.

Parel, Bombay,
20th September 1940.

S. S. SOKHEY,
Lieut.-Colonel, I.M.S.,
Director, Haffkine Institute.

PART III.

APPENDIX.

Statistics of Anti-Rabic Inoculation.

(Standard Tables approved for use in India.)

1938.

APPENDICES.

TABLE I.

A General Statement regarding Patients treated at the Institute and its Centres from 1922 to 1938.

Year.	Europeans.				Asiatics.				Europeans and Asiatics combined.			
	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.
1922...	64				365	4	1.04	175	429	4	0.89	175
{ Institute	7				236				243			
{ Centres	71				601	4	0.66	175	672	4	0.61	175
{ Combined												
1923...	115			59	730	10	1.37	359	845	10	1.18	418
{ Institute	38				1,521	14	0.78	260	1,559	12	0.76	260
{ Centres	153			59	2,251	22	0.97	619	2,404	22	0.91	678
{ Combined												
1924...	62	1	1.61	41	525	9	1.71	372	587	10	1.70	418
{ Institute	85				2,880	28	0.97	330	2,965	28	0.94	330
{ Centres	147	1	0.5	41	3,405	37	1.08	702	3,552	38	1.06	743
{ Combined												
1925...	42			27	583	6	1.02	219	625	6	0.96	246
{ Institute	187			5	3,569	28	0.78	393	3,756	29	0.78	398
{ Centres	229			32	4,152	34	0.81	612	4,381	34	0.79	644
{ Combined												
1926...	60			51	588	5	0.87	440	648	5	0.76	491
{ Institute	287			20	3,961	25	0.64	332	4,148	25	0.60	342
{ Centres	347			71	4,449	30	0.67	762	4,796	30	0.60	633
{ Combined												
1927...	51			37	678	6	0.88	468	729	6	0.82	505
{ Institute	303			31	3,457	25	0.64	490	4,160	25	0.61	521
{ Centres	354			68	4,535	31	0.68	958	4,889	31	0.61	1,026
{ Combined												
1928...	39			40	679	6	0.88	418	718	6	0.83	458
{ Institute	216			27	4,166	34	0.81	417	4,382	34	0.77	494
{ Centres	255			67	4,845	40	0.82	885	5,100	40	0.78	952
{ Combined												
1929...	35			43	650	5	0.77	522	635	5	0.78	565
{ Institute	286			19	4,621	43	0.93	507	4,907	43	0.89	526
{ Centres	321			62	5,271	48	0.91	1,029	5,592	48	0.89	1,091
{ Combined												
1930...	28			34	710			401	736			435
{ Institute	314	1	0.29	10	4,692	50	1.07	92	5,006	51	1.01	102
{ Centres	340	1	0.29	44	5,402	50	1.09	493	5,742	51	0.89	537
{ Combined												

1931...	Institute	41			42	1,020	3	0-29	394	1,031	3	0-28	436
	Centres	328			3	5,670	40	0-70	80	5,998	40	0-89	88
	Combined	369			45	6,690	43	0-64	474	7,069	43	0-60	519
1932...	Institute	40			52	1,207	3	0-24	457	1,247	3	0-24	509
	Centres	251			8	5,984	60	1-00	35	6,236	60	0-96	43
	Combined	292			60	7,191	63	0-87	492	7,483	63	0-84	552
1933...	Institute	59			62	1,535	2	0-13	722	1,594	2	0-12	784
	Centres	298			8	6,139	31	0-50	32	6,437	31	0-48	40
	Combined	357			70	7,674	33	0-43	754	8,031	33	0-41	824
1934...	Institute	45			49	1,785	4	0-22	665	1,830	4	0-21	714
	Centres	344			24	5,462	23	0-42	21	5,806	23	0-39	45
	Combined	389			73	7,247	27	0-37	686	7,636	27	0-35	759
1935...	Institute	70			64	2,202	4	0-18	593	2,272	4	0-17	657
	Centres	306			7	5,544	32	0-57	44	5,860	32	0-54	41
	Combined	376			71	7,746	36	0-46	637	8,132	36	0-44	708
1936...	Institute	81			55	2,250	2	0-08	567	2,331	2	0-08	622
	Centres	246			10	5,515	29	0-52	28	5,761	29	0-50	38
	Combined	327			65	7,765	31	0-39	595	8,092	31	0-38	660
1937...	Institute	121			53	2,892	3	0-1	420	3,013	3	0-1	473
	Centres	255			8	6,590	45	0-6	34	6,845	45	0-6	42
	Combined	376			61	9,482	48	0-5	454	9,858	48	0-4	515
1938...	Institute	58			44	2,681	1	0-04	267	2,739	1	0-04	304
	Centres	413			20	7,028	26	0-37	75	7,443	26	0-35	95
	Combined	473			64	9,709	27	0-28	335	10,182	27	0-27	399
Grand Total ...		5,176	2	0-04	953	98,415	604	0-6	10,662	103,591	606	0-5	11,615

TABLE II.
A General classification of Patients attending the Institute and its centres during 1938.
Institute.

	Europeans.					Asiatics.				
	Class of case treated.					Class of case treated.				
	I	II	III	IV	Total.	I	II	III	IV	Total.
Number fully treated ...	33	5	20	...	58	38	152	2,379	112	2,681
Percentage to total ...	56.9	8.7	34.4	...	...	1.4	5.7	88.7	4.2	...
Total deaths ...	...	...	...	...	...	...	...	1	...	1
Total death rate per cent. ...	...	...	...	...	...	...	...	0.04	...	0.04
Percentage of health returns ...	...	...	...	...	100					96.2
Number not fully treated (presumably at risk) ...	...	...	...	...	...					0
Advice cases (not at risk) ...	...	...	...	...	44					260
Cases excluded from the statistical tables for other reasons ...	...	...	...	...	...					...
Total ...					44					260
Grand Total ...					102					2,941

TABLE II (a).

A general classification of patients attending the Centres during 1938.

	Europeans.						Asiatics.				
	Class of case treated.						Class of case treated.				
	I	II	III	IV	Total.		I	II	III	IV	Total.
Number fully treated ...	316	39	39	21	415		503	981	4,115	1,429	7,028
Percentage to total ...	76.14	9.40	9.40	5.06	...		7.2	13.9	58.6	20.3	...
Total deaths ...	...	...	...	...	...		...	1	5	20	26
Total death rate per cent. ...	...	...	...	...	...		...	0.1	0.12	1.4	0.37
Percentage of health returns ...	...	...	...	...	90.3						82.2
Number not fully treated (presumably at risk) ...	...	...	...	...	...						1,386
Advice cases (not at risk) ...	...	...	...	...	20						75
Cases excluded from the statistical tables for other reasons ...	...	...	...	...	...						...
Total ...					20						1,461
Grand Total ...					435						8,489

TABLE III.

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators = Deaths.

Denominators = Cases.

Institute.

A—Animal proved rabid.

B—Animal certified rabid.

C—Animal probably rabid according to history.

D—History of animal unknown.

E—Animal probably not rabid according to history.

	Europeans.							Asiatics.							Total	Death rate per cent.
	A	B	C	D	E	Total.	Death rate per cent.	A	B	C	D	E	Total.	Death rate per cent.		
Bitten on face	...	...	...	...	...	...	...	...	...	8	70	...	78	...	78	...
Bitten on trunk and extremities (bare skin).	1	...	4	15	...	20	...	...	9	1/586	1,528	...	1/2,123	...	1/2,143	0.04
Bitten on trunk and extremities (through clothing).	1	...	...	4	...	5	...	...	12	87	343	...	442	...	447	...
"Licks" on any part of the body.	21	9	3	...	...	33	...	27	10	1	...	...	38	...	71	...
Total ...	23	9	7	19	...	58	...	27	31	1/682	1,941	...	1/2,681	...	1/2,739	...
Percentage to total ...	39.7	15.5	12.0	32.8	...	...	...	1.0	1.1	25.4	72.5	...	97.1	...	...	...
Death rate ...	...	...	...	...	...	...	...	...	...	0.1	...	...	0.04	...	0.04	...

TABLE III (a).

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators = Deaths.

Denominators = Cases.

Centres.

A—Animal proved rabid.

B—Animal certified rabid.

C—Animal probably rabid according to history.

D—History of animal unknown.

E—Animal probably not rabid according to history.

	Europeans.							Asiatics.							Total.	Death rate per cent.
	A	B	C	D	E	Total.	Death rate per cent.	A	B	C	D	E	Total.	Death rate per cent.		
Bitten on face ...	...	...	2	...	...	2	...	2	2/15	12/812	56	...	14/335	3.6	14/337	3.6
Bitten on trunk and extremities (bare skin).	...	4	20	18	...	42	...	...	178	11/3,095	891	...	11/4,164	0.2	11/4,206	0.3
Bitten on trunk and extremities (through clothing).	...	...	14	8	...	22	...	...	...	1/1,169	897	...	1/2,066	0.05	1/2,088	0.05
"Licks" on any part of the body.	80	42	119	108	...	349	...	68	118	186	41	...	413	...	762	...
Total ...	80	46	155	134	...	415	...	70	2/311	24/4,762	1,885	...	26/7,028	0.37	26/7,443	...
Percentage to total ...	19.3	11.1	37.3	32.3	...	...	...	0.1	4.5	67.8	26.7	...	...	...	...	...
Death rate ...	...	...	...	...	...	...	...	...	0.6	0.6	...	...	...	0.37	...	0.35

TABLE IV.

Age and sex distribution of patients treated at the Institute.

Numerator = Deaths.					Institute.				Denominators = Cases.	
Age in years.	Europeans.				Asiatics.				Europeans and Asiatics combined.	
	Males.	Females.	Total.	Death rate per cent.	Males.	Females.	Total.	Death rate per cent.	Total.	Death rate per cent.
0—4 ...	6		6		77	43	120		126	
5—9 ...	2	6	8		289	90	379		387	
10—14 ...	3	1	4		311	59	370		374	
15—19 ...	2	2	4		206	32	238		242	
20—29 ...	8	12	20		549	84	633		653	
30—39 ...	5	4	9		1/410	75	1/485	0·2	1/494	0·2
40—49 ...	3	1	4		186	64	250		254	
50—59 ...		1	1		89	36	125		126	
60—69 ...	2		2		42	30	72		74	
70 and over ...					8	1	9		9	
Total ...	31	27	58		1/2,167	514	1/2,681	0·04	1/2,739	0·04

TABLE IV (a).

*Age and sex distribution of patients treated at the Centres.**Numerator = Deaths.**Denominators = Cases.*

Age in years.		Europeans.*				Asiatics.				Europeans and Asiatics combined.	
		Males.	Females.	Total.	Death rate per cent.	Males.	Females.	Total.	Death rate per cent.	Total.	Death rate per cent.
0—4	...	10	6	16		1/163	3/121	4/284	1.4	4/300	1.3
5—9	...	21	23	44		4/802	1/251	5/1053	0.5	5/1097	0.5
10—14	...	23	12	35		3/806	298	3/1104	0.3	3/1139	0.3
15—19	...	14	17	31		418	154	572		603	
20—29	...	131	30	161		3/1,815	2/487	5/2302	0.2	5/2,463	0.2
30—39	...	37	28	65		2/613	1/213	3/826	0.4	3/891	0.3
40—49	...	31	19	50		2/365	1/169	3/534	0.6	3/584	0.5
50—59	...	6	5	11		2/200	1/59	3/259	1.2	3/270	1.1
60—69	...	1	1	2		52	18	70		72	
70 and over	...			...		18	6	24		24	
Total	...	274	141	415		17/5,252	9/1,776	26/7,028	0.37	26/7,443	0.35

TABLE V.

Classification according to number and position of wounds.

Europeans.

Institute.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg ...	...	2	3	3	2	1	1	...	...	...	...	...	1	...	...	...	13	...
Trunk ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Arm ...	...	33	4	3	1	1	2	...	1	...	...	...	...	...	...	...	45	...
Multiple	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Face ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Multiple face	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	33	6	6	4	3	3	1	1	...	...	...	...	1	...	...	...	58	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics : Also for Institute and Centres :—

(V) Europeans—Institute.

(V-a) „ —Centres.

(V-b) Asiatics —Institute.

(V-c) „ —Centres.

TABLE V-a.
Classification according to number and position of wounds.

Europeans.

Centres.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg ...	...	11	8	3	5	...	...	...	...	...	...	...	...	...	...	1	28	...
Trunk ...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	1	...
Arm ...	349	14	4	7	2	1	...	...	1	...	...	...	...	1	...	...	379	...
Multiple	...	...	...	1	...	...	1	1	...	...	...	...	...	...	...	1	4	...
Face ...	...	...	1	1	...	...	...	...	...	...	...	...	...	...	...	...	2	...
Multiple face	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	1	...
Total ...	349	25	13	13	7	2	1	1	1	...	...	...	...	1	...	2	415	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics : Also for Institute and Centres:—

(V) Europeans—Institute.
(V-a) „ —Centres.
(V-b) Asiatics —Institute.
(V-c) „ —Centres.

TABLE V-b.

Classification according to number and position of wounds.

Numerators = Deaths.

Denominators = Cases.

Asiatics.
Institute.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg	...	264	692	318	293	105	64	42	13	5	5	7	1	...	...	1	1810	
Trunk	...	8	21	4	4	4	3	2	...	...	...	...	...	...	...	...	46	
Arm	38	110	216	103	99	38	27	17	1/14	1	3	2	3	...	1	4	1/676	0.1
Multiple	...	...	3	6	11	11	9	8	7	4	4	2	1	1	1	3	71	
Face	...	14	28	6	13	2	3	2	...	1	...	...	...	...	...	...	69	
Multiple face	...	...	...	...	2	1	2	1	...	2	...	...	...	...	1	...	9	
Total	38	396	960	437	422	161	108	72	1/34	13	12	11	5	1	...	8	1/2681	
Percentage death rate.	...	...	...	...	...	...	...	...	2.9	...	...	...	...	...	...	...	0.04	

Separate table for Europeans and Asiatics : Also for Institute and Centres :—

(V) Europeans—Institute.

(V-a) „ —Centres.

(V-b) Asiatics—Institute.

(V-c) „ —Centres.

TABLE V-c.

Classification according to number and position of wounds.

Numerators=Deaths.

Denominators=Cases.

Asiatics.
Centres.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Per- centage death rate.
Leg	...	697	1226	984	4/644	188	146	47	45	16	11	6	8	3	7	3	4/4031	0.1
Trunk	...	44	93	85	25	17	11	4	4	1	...	...	1	...	...	...	285	-
Arm	413	2/271	1/476	2/333	2/273	1/73	71	30	34	8	10	2	6	3	2	6	8/2011	0.4
Multiple	...	...	21	27	67	42	49	34	30	13	4	7	5	2	2	13	316	...
Face	...	2/60	3/63	59	4/39	11	2	4	1/8	1/3	1/3	...	...	...	...	2	12/254	4.7
Multiple face	...	...	9	1/15	27	13	20	9	12	7	4	3	3	3	1	1/5	2/131	1.5
Total	413	4/1072	4/1888	3/1503	10/1075	1/344	299	128	1/133	1/48	1/32	18	23	11	12	1/29	26/7028	...
Percentage death rate.	...	0.4	0.2	0.2	0.9	0.3	...	...	0.7	2.1	3.1	...	...	...	...	3.4	0.37	...

Separate table for Europeans and Asiatics; Also for Institute and Centres.

(V) Europeans—Institute.
 (Va) „ —Centres.
 (Vb) Asiatics—Institute.
 (Vc) „ —Centres.

Europeans.

Institute.

[illegible]

TABLE VI-a.

Classification according to number and depth of wounds and interposition of clothing.

Europeans.

Centres.

[illegible]

TABLE VI-b.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths.

Asiatics.

Denominators = Cases.

Institute

[illegible]

TABLE VI-c.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths.

Asiatics.

Denominators = Cases.

Centres.

Deep Bare.	Number of wounds.															Total	Per- cent- age death rate.	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14			15 and over
+ +	...	3/468	3/1014	3/991	7/621	174	147	61	1/66	1/21	1/14	4	13	7	5	1/15	20/3621	0.6
+ -	...	243	1/444	315	3/311	1/124	107	50	49	22	17	12	9	4	7	11	5/1725	0.3
- +	413	1/241	264	110	72	26	28	12	11	2	1	1	...	...	...	2	1/1183	0.08
- -	...	120	166	87	71	18	19	5	7	3	...	1	1	...	...	1	493	...
Total...	413	4/1072	4/1888	3/1503	10/1075	1/344	299	123	1/133	1/48	1/32	18	23	11	12	1/29	26/7028	...
Per- cent- age death rate.	...	0.4	0.2	0.2	0.9	0.3	...	...	0.7	2.1	3.1	...	...	...	...	3.4	0.37	...

Europeans.

[illegible]

TABLE VII-a.

Classification according to number of wounds and lateness of arrival.

Europeans.

Centres.

Days late.		Number of wounds.															Total	Per- cent- age death rate.	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14			15 and over
0	...	79	4	...	1	1	...	...	...	...	...	...	...	...	...	...	1	86	...
1	...	112	5	6	7	3	...	1	...	...	...	...	...	...	1	...	1	136	...
2	...	34	5	2	2	2	...	...	...	...	...	...	...	...	...	...	...	45	...
3	...	18	3	2	1	1	1	...	...	1	...	...	...	...	...	...	...	27	...
4	...	30	5	2	1	...	1	...	1	...	...	...	...	...	...	...	...	40	...
5	...	19	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	19	...
6	...	4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	...
7	...	10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	10	...
8 to 14	...	25	1	1	1	...	...	...	...	...	...	...	...	...	...	...	...	28	...
15 to 21	...	6	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	7	...
22 to 28	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
29 to 35	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	...
36 to 42	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	...
43 to 49	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	...
50 and over	...	8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	8	...
Total	...	349	25	13	13	7	2	1	1	1	...	...	...	...	1	...	2	415	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VII-c.

Classification according to number of wounds and lateness of arrival.

Numerators=Deaths.

Asiatics.

Denominators=Cases.

Centres.

Days late.	Number of wounds.																Total.	Per- cent- age death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.		
0	75	1/93	176	95	92	26	36	14	12	4	2	3	3	2	...	3	1/636	0.15
1	55	1/210	459	1/404	251	80	77	30	45	1/13	8	5	4	3	1	1/15	4/1660	0.24
2	78	205	261	310	3/214	65	56	21	1/30	10	5	3	5	2	3	6	4/1374	0.39
3	29	190	287	1/254	1/156	56	31	18	21	5	1/7	4	4	1	1	1	3/1065	0.28
4	45	1/123	1/200	143	4/133	33	36	13	15	7	6	...	3	2	2	1	6/762	0.8
5	18	44	1/91	87	1/54	27	16	8	3	2	...	2	2	...	2	1	2/357	0.6
6	23	45	65	39	1/27	7	11	6	2	1	1	...	1	...	1	...	1/229	0.4
7	10	1/13	1/36	19	22	5	3	4	...	...	1	...	...	...	2	...	2/115	1.7
8 to 14	36	90	1/152	1/90	78	37	17	9	4	4	1	1	1	1	...	1	2/502	0.4
15 to 21	14	29	43	29	23	1/4	9	4	...	1	...	...	...	...	...	...	1/156	0.6
22 to 28	1	5	7	4	4	1	1	...	...	...	...	...	...	...	...	...	23	...
29 to 35	13	10	10	12	7	1	2	1	1	1	1	...	...	...	...	1	60	...
36 to 42	5	3	4	...	1	...	2	...	...	...	...	...	...	...	...	...	15	...
43 to 49	...	3	5	8	8	...	2	...	...	...	...	...	...	...	...	...	26	...
50 and over	11	9	12	9	5	2	...	...	...	...	...	...	...	...	...	...	48	...
Total	413	4/1072	4/1388	3/1503	10/1075	1/344	299	128	1/133	1/48	1/32	18	23	11	12	1/29	26/7028	
Percentage death rate.	...	0.4	0.2	0.2	0.9	0.3	...	...	0.7	2.1	3.1	...	...	...	...	3.4	0.37	...

TABLE VIII.

Classification according to position and depth of wounds, and interposition of clothing.

Europeans.

Institute.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+ + ...	6	...	11	...	...	...	17	...
+ - ...	3	...	...	...	...	...	3	...
- + ...	2	...	34	...	...	...	36	...
- - ...	2	...	...	...	...	...	2	...
Total ...	13	...	45	...	...	...	58	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE VIII-a.

Classification according to position and depth of wounds, and interposition of clothing.

Europeans.

Centres.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+ + ...	6	...	9	3	...	1	19	...
+ - ...	7	...	5	1	1	...	14	...
- + ...	8	1	362	...	...	...	371	...
- - ...	7	...	3	...	1	...	11	...
Total ...	28	1	379	4	2	1	415	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE VIII-b.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators = Deaths.

Asiatics.

Denominators = Cases.

Institute.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+ + ...	1396	5	1/541	43	53	8	1/2051	0.05
+ - ...	229	29	27	18	1	...	364	...
- + ...	63	4	103	7	10	1	188	...
- - ...	62	8	5	3	...	...	78	...
Total ...	1,810	46	1/676	71	69	9	1/2681	...
Percentage death rate.	...	...	0.1	...	...	...	0.04	...

TABLE VIII-c.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators = Deaths.

Asiatics.

Denominators = Cases.

Centres.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+ + ...	4/2167	139	6/342	173	8/135	2/65	20/3621	0.6
+ - ...	1,049	73	1/364	100	4/85	48	5/1725	0.3
- + ...	512	43	1/578	23	19	8	1/1183	0.08
- - ...	303	24	127	20	15	10	499	...
Total ...	4/4031	285	8/2011	316	12/254	2/131	26/7028	...
Percentage death rate.	0.1	...	0.4	...	4.7	1.5	0.37	...

TABLE IX.

Classification according to position of wounds and lateness of arrival.

Europeans.

Institute.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	4	...	3	...	...	...	7	...
1 ...	5	...	7	...	...	...	12	..
2 ...	1	...	2	...	...	...	3	...
3 ...	1	...	2	...	...	...	3	...
4 ...	1	...	19	...	...	...	20	...
5 ...	...	...	2	...	...	...	2	...
6 ...	...	...	4	...	...	...	4	...
7 ...	1	...	...	...	...	...	1	...
8 to 14 ...	...	...	5	...	...	...	5	...
15 to 21 ...	...	...	...	...	...	...	...	...
22 to 28 ...	...	...	...	...	...	...	...	...
29 to 35 ...	...	...	1	...	...	...	1	...
36 to 42 ...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...
50 and over .	...	...	...	...	...	...	...	...
Total ...	13	...	45	...	...	...	58	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE IX-(a).

Classification according to position of wounds and lateness of arrival.

Europeans.

Centres.

[illegible]

TABLE IX-(b).

*Classification according to position of wounds and lateness of arrival.**Numerators = Deaths.**Denominators = Cases.*

Asiatics.

Institute.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0	338	7	102	20	13	5	485	...
1	833	28	1/310	39	36	4	1/1250	0.08
2	196	3	66	5	10	...	280	...
3	73	1	35	1	3	...	113	...
4	99	4	65	2	...	...	170	...
5	41	...	22	...	1	...	64	...
6	46	...	13	...	1	...	60	...
7	21	...	8	...	...	...	29	...
8 to 14	90	1	32	2	3	...	128	...
15 to 21	29	...	12	2	2	...	45	...
22 to 28	8	...	1	...	...	...	9	...
29 to 35	12	...	4	...	...	...	16	...
36 to 42	2	1	3	...	...	...	6	...
43 to 49	4	...	1	...	...	...	5	...
50 and over	18	1	2	...	...	...	21	...
Total	1,810	46	1/676	71	69	9	1/2681	...
Percentage death rate.	...	...	0.1	...	...	...	...	0.04

TABLE IX-(c).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths.

Denominators = Cases.

Asiatics.

Centres.

Days late.		Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0	...	337	19	210	32	1/25	13	1/636	0.15
1	...	972	75	1/426	95	1/60	2/32	4/1660	0.24
2	...	1/769	53	392	71	3/57	32	4/1374	0.39
3	...	653	35	1/257	54	2/47	19	3/1065	0.28
4	...	2/411	38	2/257	29	2/17	10	6/762	0.8
5	...	206	19	1/106	10	1/11	5	2/357	0.6
6	...	1/123	14	74	7	7	4	1/229	0.4
7	...	58	9	1/40	2	1/4	2	2/115	1.7
8 to 14	...	322	12	1/133	13	1/18	4	2/502	0.4
15 to 21	...	96	6	1/48	1	2	3	1/156	0.6
22 to 28	...	13	1	6	...	2	1	23	...
29 to 35	...	30	1	21	1	2	5	60	...
36 to 42	...	8	1	10	1	...	...	15	...
43 to 49	...	16	...	8	...	2	...	26	...
50 and over	...	22	2	23	...	...	1	48	..
Total		4/4031	285	8/2011	316	12/254	2/131	26/7028	...
Percentage death rate.		0.1	...	0.4	...	4.7	1.5	0.37	...

TABLE X.

Deaths from Hydrophobia amongst persons treated in

Europeans.

Institute.

Serial number.	Case number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
Nil																					

TABLE X-a.

Deaths from Hydrophobia amongst persons treated in

Europeans.

Centres.

Serial number.	Case number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
Nil																					

TABLE X-b.

Deaths from *Hydrophobia* amongst persons treated in

Asiatics.

Institute.

Serial number.	Case number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.							Number and position of wounds.							Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Type.	Duration in days.	Treatment.
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncharacterised.														
1	1726	3	F.	32	Jackal	C	...	...	4	...	4	+	+	+	+	+	+	+	+	42	3	48	III	5 cc 5 %.	3 days.				
2	1551	13	M.	8	Dog	C	...	...	2	2	4	+	+	+	+	+	+	+	+	15	3	31	IVF	7 cc "	13 "				
1	1467	27	M.	35	Dog	C	...	...	8	...	8	+	+	+	+	+	+	+	+	1	2	30	III	5 cc "	14 "				

Died during treatment.

Died within 15 days after completion of treatment.

TABLE X-c.

TABLE
Deaths from *Hydrophobia*

Asia

Cen

Serial No.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and of wo		
					Kind.	Class.	Leg.	Trunk.	Arm.
1	596	12	M.	25	Dog	...	C	...	...
<i>Died during</i>									
1	390	14	M.	11	Dog	...	C	...	...
<i>Died within 15 days</i>									
2	25	16	M.	25	Jackal	...	C	4	...
3	96	16	M.	38	Do.	...	C	...	2
4	148	17	F.	38	Dog	...	C	...	...
5	113	17	F.	55	Wolf	...	C	...	...
6	143	18	M.	5	Dog	...	B	...	...
7	525	20	M.	22	Jackal	...	C	...	...
8	61	21	M.	4	Dog	...	B	...	...
9	489	21	M.	35	Do.	...	C	...	1
10	154	22	M.	8	Jackal	...	C	...	...
11	111	24	F.	25	Wolf	...	C	...	...
12	109	25	F.	40	Jackal	...	C	...	...
13	145	27	M.	10	Do.	...	C	1	...
<i>Died after 15 days</i>									
1	58	15	F.	25	Dog	...	C	...	5
2	55	26	M.	5	Do.	...	C	...	...
3	194	33	M.	45	Jackal	...	C	...	...
4	23	41	F.	24	Dog	...	C	...	...
5	335	42	M.	50	Do.	...	C	...	4
6	167	44	M.	43	Do.	...	C	...	6
7	147	49	M.	7	Do.	...	C	...	3
8	117	51	M.	12	Jackal	...	C	4	...
9	112	56	M.	50	Wolf	...	C	4	...
10	85	71	F.	24	Dog	...	C	...	...
11	218	97	F.	6	Jackal	...	C	...	3
12	3	101	M.	20	Dog	...	C	...	1
13	116	106	F.	2	Jackal	...	C	4	...

X-c.

amongst persons treated in

tics.

tres.

position unds.		Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
<i>treatment.</i>											
8	8	+	-	+	+	4	1	17	IVf	10 cc.	5 % 12
<i>after completion of treatment.</i>											
...	4	+	-	+	+	8	1	23	IVe	7 cc.	5 % 14
...	4	+	-	+	+	2	4	22	III	5 ..	5 .. 14
...	2	+	-	+	+	4	3	23	III	5 ..	5 .. 14
1	1	+	-	+	+	0	2	20	IVf	10 ..	5 .. 14
2	2	+	+	+	+	5	2	24	IVf	10 ..	5 .. 14
4	4	+	+	+	+	2	2	22	IVf	7 ..	5 .. 14
4	4	+	-	+	+	2	1	23	IVf	10 ..	5 .. 14
1	1	+	-	+	+	4	2	27	IVf	7 ..	5 .. 14
...	1	+	-	+	+	7	1	29	IVe	7 ..	5 .. 14
9	9	+	-	+	+	1	2	25	IVf	7 ..	5 .. 14
4	4	+	+	+	+	4	2	30	IVf	10 ..	5 .. 14
4	4	+	+	+	+	3	2	30	IVf	7 ..	5 .. 14
2	4	+	-	+	+	1	2	30	IVf	7 ..	5 .. 14
<i>after completion of treatment.</i>											
...	5	+	-	-	+	20	1	36	IVe	7 cc.	5 % 14
2	2	+	-	+	+	7	2	35	IVf	10 ..	5 .. 14
10	10	+	-	+	+	3	3	39	IVf	10 ..	5 .. 14
2	2	+	-	+	+	8	2	51	IVf	7 ..	5 .. 14
...	4	+	-	+	+	5	2	49	IVe	7 ..	5 .. 14
13	19	+	-	+	+	1	2	47	IVf	10 ..	5 .. 14
...	3	+	-	+	+	4	2	55	IVe	5 ..	5 .. 14
...	4	+	-	+	+	2	2	55	III	5 ..	5 .. 14
...	4	+	-	+	+	4	3	63	III	5 ..	5 .. 14
8	8	+	-	+	+	2	2	75	IVf	7 ..	5 .. 14
...	3	+	-	+	+	3	2	102	IVe	7 ..	5 .. 14
...	1	-	-	+	+		2	104	II	2 ..	5 .. 14
...	4	+	-	+	+	6	2	114	III	35 ..	5 .. 14

TABLE

Classification of the animal which bit,

Numerators = Deaths.

Animal.	INSTITUTE.								
	Number treated.			Percentage to total.			Death rate per cent.		
	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.
Dog ...	53	1/2,534	1/2,587	91.5	94.5	94.4	.	0.04	0.04
Jackal ...	2	31	33	3.4	1.2	1.2	.	...	...
Wolf ...	...	2	2	...	0.07	0.07	.	...	...
Cat ...	2	42	44	3.4	1.6	1.6	...	...	...
Monkey ...	...	63	63	...	2.4	2.3	.	...	...
Fox ...	...			...	...	...	...	...	...
Human beings ...	...			...	...	...	...	...	...
Tiger ...	...			...	...	...	.	...	...
Pig ...	...	1	1	...	0.03	0.03	.	...	...
Mongoose ...	1	1	2	1.7	0.07	0.07	.	.	...
Goat ...	...			...	...	...	...	...	...
Buffalo ...	...			...	...	...	...	...	...
Bullock ...	...			...	...	...	...	...	...
Cow ...	...	1	1	...	...	0.03	...	...	...
Horse ...	...	3	3	...	...	0.1	...	...	...
Ass ...	...	1	1	...	...	0.03	...	...	...
Deer ...	...	1	1	...	...	0.03	...	...	...
Bear ...	...	1	1	...	...	0.03	...	...	...
Total ...	58	1/2,681	1/2,739	...	...	...	...	0.04	0.04

XI.

*scratched or licked the patient.**Denominators—Cases.*

CENTRES.								
Number treated.			Percentage to total.			Death rate per cent.		
Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.
345	13/6043	13/6388	83.3	85.9	85.8		0.21	0.20
.....	10/793	10/793	...	11.2	10.8		0.12	0.12
...	3/22	3/22		0.3	0.3		13.6	13.6
.....	14	14		0.2	0.2		...	...
5	19	24	1.1	0.3	0.3		...	...
.....	5	5		0.07	0.07		...	...
65	52	117	15.6	0.7	1.6		...	...
.....	1	1		0.015	0.015		...	...
.....	1	1		0.015	0.015		...	...
.....	2	2		0.03	0.03		...	...
.....	4	4		0.06	0.05		...	...
.....	6	6		0.09	0.08		...	...
.....	1	1		0.015	0.015		...	...
.....	2	2		0.03	0.03		...	...
.....	47	47		0.7	0.7		...	...
.....	16	16		0.2	0.2		...	...
.....	...		...	...	...		...	...
.....	...			...	...		...	...
415	26/7028	26/7443		...	...		0.37	0.35

TABLE XII.

Number of patients treated by months.

	Institute.			Centres.			Institute and Centres combined.		
	Euro-peans.	Asiatics.	Total.	Euro-peans.	Asia-tics.	Total	Euro-peans.	Asia-tics.	Total.
January ...	...	254	254	41	560	601	41	814	855
February ...	2	240	242	33	528	561	35	768	803
March ...	4	296	300	44	693	737	48	989	1,037
April ...	3	262	265	27	652	679	30	914	944
May ...	12	253	265	23	642	665	35	895	930
June ...	5	213	218	21	546	567	26	759	785
July ...	10	193	203	94	533	627	104	726	830
August ...	3	213	216	29	652	681	32	865	897
September ...	13	184	197	38	538	576	51	722	773
October ...	1	163	164	19	538	557	20	701	721
November ...	2	180	182	25	566	591	27	746	773
December ...	3	230	233	21	580	601	24	810	834
Total ...	58	2,681	2,739	415	7,023	7,443	473	9,709	10,182

TABLE XIII.

Statement showing brains submitted and examined for rabies.

Serial number.	Animal.	Number of brains examined.	Positive.	Negative.	Unfit for examination.
1	Dogs ...	152	108	41	3
2	Buffalo ...	6	3	3	
3	Jackals ...	2	2	...	
4	Bullocks ...	2	...	2	
5	Horse ...	2	1	1	
6	Donkey ...	2	1	1	
7	Deer ...	1	...	1	
	Total ...	167	115	49	8

