



GOVERNMENT OF MAHARASHTRA

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HAFFKINE INSTITUTE**

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Deputy Directors

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Deputy Director (Research), Division of Pharmacology and Pharmaceutical
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Dr. S. S. Rao, M.Sc. (Banaras), Ph.D. (Toronto),
Department of Immunology.

Dr. K. V. Kulkarni, M.B.B.S. (Bom.),
Department of Immuno-haematology.

Shri Y. S. Nimbkar, M.Sc. (Bom.), Dip. (Bact.) (Manchester),
Department of Bacteriology (upto 30-7-1971).

Dr. F. L. Saldanha, Ph.D. (Bom.),
Department of Analytical and Control (from 19-4-1971).

Shri K. G. Anantanarayanan, M.Sc.,
Department of Analytical and Control (upto 18-4-1971).

(B)

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Y. V. Thakre, M.A., LL.B. (Nagpur)
(from 12-10-1971).

Dr. K. H. Dave, M.D. (Bom.),
Department of Viral Vaccines.

Shri M. E. Kulkarni, M.Sc., Dip. (Bact.),
Department of Bacteriology (from 31-7-1971)

Dr. (Smt.) J. K. Dave, M.D. (Bom.),
Department of Clinical Pathology (from 2-1-1972).

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PART I
GENERAL

GENERAL REVIEW

Haffkine Institute is one of the oldest research institutes in the country. It is involved in advanced research in communicable diseases. The Institute was established in 1896 as a Bacteriological Research Laboratory under the Directorship of Dr. W. M. Haffkine. Since then, the Institute has evolved into a premier research institute of the country. It has about 10 research departments in addition to a library, maintenance engineering section and an animal farm. The Institute employs about 1,400 persons including about 200 junior and senior scientists including technicians.

The Institute performs 3 important functions in addition to rendering public health services. Its principal objective is to carry on extensive research of a very high order in communicable diseases in all its aspects. In addition to this, the Institute has been involved, right from its inception, in the production of a number of lifesaving drugs, especially biologicals. In fact, since Dr. Haffkine discovered plague vaccine, he was called upon quite rightly to prepare the vaccine on a large scale, so as to distribute it throughout the country for fighting the dreadful scourge of plague. Since then, the Institute has undertaken production of a number of biological preparations, such as vaccines, sera and toxoids. Since the Second World War, the Institute also started research development in the field of chemotherapeutic agents and has been manufacturing a number of pharmaceuticals, such as vitamins, anti-T.B. drugs, etc., specifically for the requirements of the State Government, hospitals and dispensaries. Although, production is not the major activity of the Institute, nevertheless, it is manufacturing a large number of drugs and supplies these at "No profit no loss basis" to State Governments, Defence Services and such other official agencies or institutions.

Haffkine Institute is a training ground for a number of scientists. The Institute has, on its roll, about 74 students who have registered for M.Sc. or Ph.D. degrees in various branches such as microbiology, biochemistry, applied biology, organic chemistry and zoology. The Institute is affiliated to the University of Bombay. It also conducts courses for training in medical laboratory technology. Thus, it admits about 12 students every year for a diploma course in Medical Laboratory Technology (D.M.L.T.) offered by the University of Bombay and, in addition, conducts a course in Medical Laboratory Technicians' Training and offers its own certificate to successful candidates.

The Institute renders public health laboratory service. This serves not only to establish a liaison between the Institute and the public, but its interest in communicable diseases is also further strengthened. At short notice, it sends its expert to any part of the city for 'on the spot' investigation. It undertakes drug testing and gives expert opinion on clinical specimens referred to the Institute from different parts of the State of Maharashtra, especially from the suburban and rural areas.

One of the very important aspects of research developments in the Institute is in the field of epidemiology. The Institute has undertaken epidemiological studies in different diseases prevalent in the State of Maharashtra in close collaboration with the Public Health Department of the State. It is involved in epidemiological research in gastro-enteritis and filariasis.

In the last year, the country was involved in a national emergency and the Institute rose to the occasion and geared up its production activity to fulfil the demands of the armed forces for biologicals. It also did a lot of collection of blood from the voluntary donors.

Due to emergency, there was a general economic stringency and, therefore, the Institute had to rigorously implement all the steps to economise in expenditure on production and research. A number of posts, which were sanctioned and were essential had, therefore, to remain unfilled.

The Institute has continued all its research projects. These were reviewed by the Research Advisory Board. In fact, the projects were modified and the various recommendations of the Research Advisory Board, made in the previous year, were implemented.

Significant contributions in research pertain to cholera, filariasis and diphtheria. It was shown that berberine was useful in controlling choleraic diarrhoea in infant rabbit model. Attempts were made to prepare radiation-killed cholera vaccine as well as live cholera vaccine in order to increase the efficacy of the preparation. Extensive epidemiological work was carried out in filariasis in an endemic area near about Bombay. Filarial antigen was used to test the validity of the skin test in clinical cases of filaria and their contacts.

The Biochemistry Department carried out tests on the effects of various drugs on the macro-molecules of diphtheria and *Vibrio cholerae*. It was shown that aspartic acid is absolutely essential for growth of *Vibrio cholerae*, both Ogawa and Inaba and 2 El tor strains (Inaba and Ogawa). Further, drugs like furazolidine, chlormphenicol, ampicillin and berberine, were studied for their mechanism of action at a molecular level in *Vibrio*

cholerae. It was shown that all these substances inhibit the growth and affect metabolism. Ampicillin-treated cells showed decrease in all cellular constituents, while chloromycetin-treated cells showed lower content of proteins but higher percentage of R.N.A.

The Department of Chemotherapy has been involved in the synthesis of a large number of thiosemicarbazide and related derivatives, hydroxamic acid derivatives and pyrimidine and 1-hydroxypyrimidine compounds. The principal objective is to synthesize compounds which can be used in the treatment of tuberculosis and leprosy. The department has synthesized a series of compounds wherein, instead of substituted acetophenones, they have used benzyl methyl ketones and phenyl ethyl ketones with thiosemicarbazides. One of the compounds has shown a very promising antitubercular activity. Anticancer compound screening is continued, especially the Schiff-bases. The Schiff-base from 4-para. (aminophenyl)-2-5 dimethyl thiasole was found to be very active against the tumour model L 1210 and also against P 388 Lymphocytic leukemia, pyrimidine and 5-B-hydroxyethyl pyrimidine and their cyclic derivatives were screened for anti-tubercular, anti-fungal and anti-amoebic activity. Some of these compounds were also screened for antifertility activity.

The Department of Clinical Pathology has continued its studies on gonococcal urthritis. A new simple composite medium for identification of Enterobacteriaceae has been described. Ninety strains of *C. diphtheriae* were studied for toxigenicity on primary monkey kidney tissue culture medium. Studies in brucellosis in man and animals were continued and a total of 738 blood samples were screened for evidence of brucellosis.

The Institute has continued its collaborative study with the International Atomic Energy Agency on the use of labelled antigens for study of specific antibodies.

The Department of Immuno-haematology has carried out studies on genetic characters in endogamous groups of Indian Muslims. The study made on the basis of blood groups as well as G-6 PD, serum haptoglobulins and transferins and other abnormal haemoglobins, has revealed homogeneity of this group. The department has been involved in the studies on various types of immunoglobulins by starch-gel electrophoretic techniques. Especially, there is an extensive study of haptoglobins in various disease conditions, such as recurrent bacterial and viral infections, liver cirrhosis, collagen disorders and multiple myeloma. The results on the study on haptoglobins in haemolytic anaemias, cirrhosis of liver (infantile type), malnutrition and cardiac prosthesis showed a decrease in the haptoglobin levels. The department has also continued its interest in the study of

specific human hyperimmune tetanus immunoglobulins and Anti-D immunoglobulins. The department has continued its interest in Australia antigen. A new technique of counter immunoelectrophoresis, as recommended by the W.H.O., has been adopted. It was interesting to note that in normal voluntary donors, 1 per cent. showed the presence of Australia antigen, while in professional blood donors, the percentage was found to be higher. In all, 338 cases of infective hepatitis were studied and 11 per cent. were found to show presence of Australia antigen. Out of 73 cases of multiple transfusions, 10 per cent. showed presence of antibody to Australia antigen. The department has standardised methods for preparations of cryoprecipitates from fresh human plasma for the treatment of haemophilia. Studies on G-6 PD and PK deficiencies were continued. Glutathione levels were also studied. It was shown that pregnant females had a significantly lower level of glutathione. Genetical and red cell enzyme studies in *Diabetes mellitus* were carried out.

The Department of Immunology has continued its research on stability of purified antitoxic sera. Further, tetanus toxin production was studied to determine the optimum conditions for maximum toxin production. Preliminary results showed that casein digestion followed by heating to boiling at pH 4.6 gave the best results. Studies on steroid hormones on antibody production were undertaken. It was shown that ethinyl oestradiol and methandienone had a definite effect of increasing the antibody production.

Six distinct classes of immunoglobulins were identified in the buffalo sera. Studies were continued on the transfer of antibodies through colostrum to the new calf. These studies indicated that there was probably a lack of transfer of antibodies through the placenta and the antibody transfer probably occurs in the calf after ingestion of colostrum.

The Department of Pharmacology has continued its interest in the studies on cholera, filariasis, experimental amoebiasis and leprosy. It has also been screening compounds synthesised by the Chemotherapy Department for their pharmacodynamic activities. One of the compounds has been found to be a promising hypoglycaemic agent and is being extensively studied further for its toxicological properties. It has been shown that berberine is effective in cholera in antagonizing the action of cholera toxin at a receptor level. Anti-inflammatory compounds like Indomethacin and Ibuprofen were tested for their effectiveness in experimental cholera. Both these drugs were found to be ineffective. NAG strains of *Vibrio* were studied for their pathogenicity in infant cholera model and adult rabbit isolated loop. Both the strains were found to be pathogenic and produced symptoms similar to those produced by Inaba or Ogawa strains. The work on purification of cholera toxin is continued.

The Department of Viral Vaccines continued its activities vigorously. The programme of production of poliomyelitis vaccine is being undertaken. Imported polio vaccine has been distributed not only in the State but throughout the country.

The Institute has a very large centre for studies on enteroviruses financed by the Indian Council of Medical Research. The centre carries on poliomyelitis surveillance programme, both in the city and in rural areas. The Enterovirus Unit studied during the year 810 cases of paralytic poliomyelitis in Greater Bombay. Higher incidence of poliomyelitis was found to be between July and October. During 1971, 70 per cent. of the cases were recorded in children below two years and 96.6 per cent. of the total cases below 5 years. One of the interesting features was a study of paralytic disease amongst vaccinated children. One hundred and twelve paralytic polio cases were recorded in vaccines. Fifty-three individuals had received all the three doses of the live vaccine. Further studies are continued.

The Department of Virology has continued its studies on the pathogenesis of rabies as well as on the diagnostic criteria for rabies. The department also undertook an influenza surveillance programme and sero-epidemiology of measles and sub-acute sclerosing panencephalitis (SSPE). The department has studied the problem of interference between rabies fixed virus and Rous Sarcoma Virus. On the basis of its studies, it appears that the interfering factor is probably not identical with interferon, since the substance was heat and pH labile.

Studies were initiated in the State of Maharashtra to detect the lingering foci of plague infection since 1965. These studies were continued in various areas in Bombay. In all, 2470 rats were examined. *X. cheopis* was found to be predominantly present in certain areas. *X. astia* was present in others. Assessment of the susceptibility status of the oriental rat flea to DDT and other insecticides revealed a high degree of resistance. It was also found that some of the commensal rodents collected from Wards indicated the existence of anticoagulant resistant strains. Susceptibility of rats from different areas to plague infection was also studied with a standard challenge. Rats belonging to the species *Golunda ellioti*, *R. blandfordi* and *Tatera indica* showed a high susceptibility to *P. pestis*. Rats collected from the Satara District showed a high resistance to plague.

The Department of Analytical and Control has continued to its valuable assistance, both in the field of production and research. department is slowly evolving into a fullfledged quality control More than 800 samples involving more than 8000 different tests for limits for impurities, were tested.

The production activity has shown a steady progress. Difficulties of raw materials, lack of modernization and uncertainties of State Government demands have, to a certain extent, affected the production. However, during the year under review, production reached the figure of Rs. 129 lakhs as against Rs. 108 lakhs, showing a rise over that of the year.

The World Health Organization Combined Course in Epidemiology was at this Institute from 27-29th December 1971. Discussions on research problems of communicable diseases were arranged with help of different departments of this Institute.

A number of dignitaries visited the Haffkine Institute, notable amongst whom are : Dr. J. B. Shrivastav, Director-General of Health Services, of India, Smt. Pratibha D. Patil, Deputy Minister for Public Government of Maharashtra, Dr. D. Y. Gohokar, Chairman, Public Service Commission, Dr. Dhiman Barua, World Health Shri G. P. Nair, President, Indian Drug Manufacturers' Dr. A. B. Gilmour, Dy. Director, Red Cross Transfusion Frankfurt, Commonwealth National Advisory Bureau, Dr. A. A. Gray, Chairman, Wellcome Foundation Ltd., London; Shri S. K. Wankhede, for Finance, Government of Maharashtra, and Smt. Kusumtai Begum Ali Yawar Jung and Prof. P. N. Wahi, Director-General, Council of Medical Research.

The Critics Club continued its vigorous activities. A number of departmented their research papers.

During the year under review, the Library was reorganised to provide facilities to the readers. Card indices for scientific and technical research papers and theses were prepared. It also continued the activities as usual. In all, 23 research papers were submitted for and 24 research papers were published.

The maintenance Engineering Section was fully busy with the increased load and maintenance of equipment, etc. As a result of the national , this section carried out a number of urgent jobs departmentally, those connected with Blood Bank Department. in addition to jobs.

For the first time in the history of the Institute, an Internal Audit Wing created at this Institute to exercise check over the accurate maintenance various records.

The welfare activities, such as the Sports Club, Library Service, etc. and to lady employees were expanded.

During the year under review, Government sanctioned the creation of a Civil Defence Unit at this Institute and the employees participated in the training programme.

The Haffkine Institute Co-operative Credit Society continued to make satisfactory progress. It also continued to work as distributing agent of Hindustan Lever for supply of animal food to Haffkine Institute. The membership increased from 890 to 924.

The dispensary continued to serve the needs of the staff and rendered better service than in the previous year. Immunization of children of the staff against polio and whooping cough was also carried out.

During the year under review, the following officers retired :—

- (1) Dr. N. K. Dutta, Director ... November 1971
- (2) Shri K. G. Anantanarayanan, Senior Scientific Officer, Class I. November 1971
- (3) Shri V. Y. More, Senior Scientific Officer, Class II. February 1972

Shri Y. V. Thakre joined as Assistant Director (Administration) in October 1971. Dr. F. L. Saldanha took over charge as Assistant Director-in-Charge, Department of Analytical and Control, in April 1971. Shri A. Sanjiva joined as Assistant Engineer (Maintenance) on deputation, in September 1971, in place of Shri M. R. Chaudhuri repatriated. Shri M. E. Kulkarni was temporarily promoted as Assistant Director-in-Charge, Department of Bacteriology, in place of Shri Y. S. Nimbkar appointed as Deputy Director (Production).

Dr. (Smt.) J. K. Dave took over charge as Assistant Director of the Department of Clinical Pathology in February 1972.

Dr. (Smt.) S. M. Modak, Senior Scientific Officer, Class-II, went abroad on study leave.

Shri K. N. Joishy, Government of India Scholar at this Institute, was awarded the Gharpure Prize for the best paper read in the Teaching Pathologists' Meeting, during the year 1971.

Some staff from the Microbiology Section of the Pharmacology Department was transferred to the Drug Control Laboratory at Bandra (Bombay) in March 1972.

The Institute participated in the following exhibitions organised by the Director of Publicity :—

- (1) Agro-Industrial Exhibition, Yeotmal.
- (2) Agro-Industrial Exhibition, Aurangabad.

The Institute continued to attract grants from different sources, such as the International Atomic Energy Agency, Indian Council of Medical Research, Council of Scientific and Industrial Research, World Health Organization, Unichem Laboratories, Defence Authorities, etc.

Airconditioning of two major departments, viz. Immuno-haematology and the Department of Antitoxins and Sera was in progress. Another important job carried out was the construction of a cold room in the Polio Section of the Department of Viral Vaccines as also in the Toxin Section of the Department of Antitoxins and Sera. These were completed and put into commission. The construction of Class IV Quarters at Pimpri was completed, whereas construction of Class IV Quarters at Bombay is still in progress.

The Director and the senior members of the staff were greatly benefited by the advice and guidance of the Haffkine Institute State Advisory Board and the Haffkine Institute Research Advisory Board. They wish to thank all the members of the Boards for taking keen interest in the working of this Institute.

The Director and the members of the staff greatly appreciate the support they received from the administrative heads at Sachivalaya, without which many of the changes brought about in the Institute could not have been done.

The Heads of Departments and senior members of the staff significantly contributed to the different activities, be they in the field of research, production, training or teaching. Without their active co-operation, it would not have been possible to maintain the tempo of the work.

The Institute is dependent on the integrity, conscientiousness, drive and skill of the individual members of the staff and it is gratifying to note that all the members contributed to the best of their ability. The Director takes this opportunity to thank them all for the continued support he received from the administrative, scientific and non-scientific workers of the Institute.

PART II
RESEARCH

A. RESEARCH PROJECTS

B. DEPARTMENTAL RESEARCH

A. RESEARCH PROJECTS

CHOLERA PROJECT

Effect of drugs in experimental cholera

In the earlier report, Berberine was shown to be effective in infant rabbit cholera model. Since then, the mechanism of action of Berberine has been further evaluated. It is now clear that Berberine may be effective in cholera by antagonising the action of cholera toxin at a receptor, activation of which may result in the choleraic diarrhoea. Further work is in progress. Newer anti-inflammatory drugs like Indomethacin and Ibuprofen were tested for their effectiveness in experimental cholera in infant rabbits. Both the drugs delayed the onset of diarrhoea, without affecting mortality.

Study of NAG Vibrios

Three NAG strains (non-agglutinable vibrios), obtained through the courtesy of Dr. Pal of Infectious Diseases Hospital, Delhi, which caused cholera-like disease in clinical cases, were studied for their pathogenicity and antigenic character in laboratory animals.

Pathogenicity studies carried out in infant rabbits (Dutta and Habbu, 1955) and adult rabbit isolated loop technique (De and Chatterjee, 1953) showed that infant rabbits suffered from cholera-like disease. Symptoms were similar to those produced when infected with classical Inaba or Ogawa vibrios.

Adult rabbit loops isolated by using the above method showed characteristic accumulation of fluid as compared to control.

Active mouse protection test results showed that the animals immunized with cholera-EI-tor vaccine (Haffkine) challenged with *V. cholerae* 569 B Inaba or 5321 Ogawa culture were almost completely protected. However, when challenged with NAG strains, all the animals succumbed to the disease even though the mice were immunized with a high dose of vibrios.

Passive protection test in infant rabbits (Dutta *et al*, 1966), using hyperimmune antisera raised in adult rabbits against living vibrios, showed that NAG antisera gave significant protection to animals against homologous challenge. The same antisera failed to do so against heterologous challenge by *V. cholerae* Inaba 569 B and Ogawa 5321. Similarly, antisera against *V. cholerae* Inaba 569 B and Ogawa 5321 gave significant protection against homologous challenge but did not give any protection when challenged with NAG vibrios.

The above results showed that there is no cross-protection among classical vibrios and NAG vibrios. These results further confirmed the findings

reported last year, when similar experiments were carried out with NAG strains which were isolated from "Severe Gastroenteritis" cases obtained from the W.H.O.

Studies of NAG toxins and their related antigens are in progress.

Purification and characterization of cholera toxin

The work on the purification of cholera toxin was continued by using different methods. Cholera toxin prepared by growing the strain Inaba 569 B in syncase medium was passed through a column of DEAE cellulose using M/100 phosphate buffer, pH 7.5 as the eluent. Two distinct peaks were observed, the first peak contained both the choleraogenic toxin and the permeability factor, while the second peak contained only the permeability factor. The presence of only permeability factor in the second peak indicates that the permeability factor is different and can be separated from the choleraogenic toxin. The exact nature of these two fractions was determined by different enzymes. Further work on the antigenicity of these two fractions is in progress.

Study of El tor water 6 culture-filtrate antigenicity in mice

V. cholerae biotype El tor water 6 was grown in 3% peptone broth containing 10 per cent. NaCl (pH 7.6) at 37° C on a rotary shaker (100 rev./min.) for 24 hours. The broth was centrifuged for 20 minutes and sterilised by passing through Millipore filter. This was used for immunizing Kasauli mice (18 gm weight) by intraperitoneal route.

Complete protection was obtained against challenge with *V. cholerae* Ogawa 5321 and Inaba 569 B, but such immunisation gave no protection against non-agglutinable strains. Sd 280-13026, Sd 281-13030 and Sd 282-13031 received from Sudan and Cz 16/66-10125 received from Czechoslovakia.

Standardisation of cholera vaccine

Cholera vaccine is standardised presently by methods described by Sokhey and Habbu (1950) or by method described by Feeley and Pittman (1960). However, in both these methods, the syndrome produced in mice is not typical of cholera. Since infant rabbit model is a prototype of cholera as seen in patients, this has been investigated for standardisation of cholera vaccine. Rabbits were hyperimmunised by multiple intravenous doses of cholera vaccine. Antibody and vibriocidal titres were determined. The hyperimmune sera were administered to infant rabbits as such or in varying dilutions pre or post-operatively, after an intestinal challenge with live virulent *V. cholerae*. The tables 1, 2 and 3 give a data on the onset of diarrhoic symptoms, mortality and survival time in control and treated animals. It can be seen that the serum failed to protect the animals.

Preparation of lyophilised live cholera vaccine

Haffkine, as far back as 1895, used live cholera organisms as a vaccine. It was decided to reinvestigate a possibility of using live cholera vaccine to induce an immunity and to find out if the antigenicity is better than the conventional vaccine. Further, it was thought that a small number of live organisms may multiply and produce better and prolonged antigenic response.

In order to test this, lyophilised live cholera vaccine was prepared.

As reported previously, a method of freeze-drying was used. Effects of various factors on the survival of organisms during lyophilisation were studied. Virulence test of all the three strains was carried out in order to see if lyophilisation brings about any change. The antigenic property of the lyophilised vaccine was evaluated. Table 4 gives survival rate of *V. cholerae*. Number of surviving organisms was very poor. But, virulence and antigenic property remained unaltered. Further work on preparation of a live vaccine is in progress.

Mechanism of vibriocidal activity of drugs

Furazolidine was reported to inhibit the growth of two strains of *V. cholerae* by increasing the lag period. Further studies revealed that the drug interfered with protein synthesis. Major portion of the drug is associated with lipids and nucleic acids. Labelled glucose, adenine, guanine and uracil were used to study various biosynthetic processes in normal and drug-treated cells. It was shown that glycine is incorporated more into RNA fraction than in proteins, indicating that Furazolidine may be acting on same step at the translational level of protein biosynthesis. Kinetics of incorporation of nucleotides also revealed that there is increased uptake of these by drug-treated cells. Further, drug-treated cells produced an abnormal rise in the synthesis of all sub-fractions of RNA, although the base composition of the RNA, sub-fractions was not changed.

Mechanism of action of ampicillin, chloramphenicol and berberine on vibrio and *El tor* cholera

Effect of these drugs on cellular constituents of *V. cholerae* and *El tor* cholera was studied. All drugs were found to affect the cellular composition. It is interesting to note that berberine-treated cells had higher protein and lipid content.

A study of isolation and characterisation of proteins and RNA of vibrio and *El tor* cholera is under progress.

Radiation killed cholera vaccine

In the previous year, it has been reported that vaccines prepared by irradiation of *V. cholerae* 569-B were found more potent than the usual formalin-killed vaccine. Monovalent and divalent cholera vaccines prepared by irradiation by CO⁶⁰ source, gave vaccines which were more potent (6×2.79) than the standard formalin-killed vaccine. It is now proposed to study preparations of bigger lots of vaccine by this method.

Biochemical studies on growth requirement of *V. cholerae*

It was reported last year that hydrolysates of proteins, obtained from defatted cakes, gave good growth in all cases except with safflower cake. The proteins were analysed for their mineral and aminoacid content. Synthetic medium was prepared to find out the number and concentration of aminoacids for optimum growth. The study revealed that aspartic acid is very essential for the growth of *V. cholerae* and histidine and glutamic acid are needed for optimum growth. Magnesium, calcium, sulphur and chlorides are essential for growth of *V. cholerae*.

TABLE 1

Biological standardisation of cholera vaccine

Challenged with	Serum dilution	Time in hours		Delay in hours in onset of		Time lapse between diarrhoea and death
		Diarrhoea set in	Death ensued	Diarrhoea between control and test animals	Death in control and test animals	
<i>V. Cholerae</i> Inaba-569 B.	.. Original	22	32.6	6.4	7.0	10.6
	1 : 3	23.5	32.9	7.9	7.3	9.4
	1 : 9	25.4	29.0	9.8	3.4	3.6
	Control	15.6	25.6			10
<i>V. Cholerae</i> Ogawa-5321	.. Original	28.8	37.1	15	15.3	8.3
	1 : 3	18.66	37.16	4.8	15.4	18.5
	1 : 9	20.66	37.85	6.8	16	17.2
	Control	13.8	21.8			8.0

TABLE 2
Biological standardization of cholera vaccine

Expt-I-a

Challenged with	Serum dilution	Time in hours		Delay in hours in on set of		Time lapse between diarrhoea and death
		Diarrhoea set in	Death ensued	Diarrhoea between control and test animals	Death between control and test animals	
V. Cholerae	.. Original	26.8	36.1	10.2	13.7	9.3
Inaba-569-B	1:3	27.0	32.0	10.4	9.6	5.0
	1:9	19.6	24.0	3.0	1.6	4.4
	Control	16.6	22.4			5.8
V. Cholerae	.. Original	24.6	34.8	14.6	18.0	10.2
Ogawa-5321	1:3	12.0	20.6	2.0	3.9	8.6
	1:9	17.6	22.9	7.6	6.2	5.3
	Control	10.0	16.75			6.75

TABLE 3
Biological standardization of cholera vaccine

Expt-II-a

Challenged with	Serum dilution	Time in hours		Delay in hours in on set of		Time lapse between diarrhoea and death
		Diarrhoea set in	Death ensued	Diarrhoea in control and test animals	Death between control and test animals	
V. Cholerae	.. Original	22	26.6	4	4.6	4.6
Inaba-569-B	1:3	20	27.4	2	5.4	7.4
	Control	18	22.0			4.0
	Original	22	28.0	10	13.0	6.0
	1:3	16	25.4	4	10.4	9.4
	Control	12	15.0			3.0

TABLE 4

Percentage survival of V. cholerae strains after freezing and lyophilization

Organisms	Suspending medium	No. of organisms/ml. before processing X 10 ⁶		No. of organisms/ml. after freezing only. X 10 ⁶		No. of organisms/ml. after lyophilization. X 10 ⁶	
V. Cholerae	6 % lactose + 6 % sodium glutamate.	65000	89500	4050	10600	12	12
El tor water 6		(N.A.)	(B.A.)	(N.A.) (6·23)	(B.A.) (11·84)	(N.A.) (0·019)	(B.A.) (0·047)
		8150 (N.A.)	11100 (N.A.)	3000 (N.A.) (36·81)	5250 (B.A.) (47·3)	16 (N.A.) (0·196)	41·6 (B.A.) (0·375)
V. Cholerae	6 % lactose + 6 % sodium glutamate.	27000	77500	1566	8050	65	58·5
Ogawa C ₁₄ — S ₅		(N.A.)	(B.A.)	(N.A.) (5·8)	(B.A.) (10·4)	(N.A.) (0·241)	(B.A.) (0·755)
		4525 (N.A.)	16600 (B.A.)	1810 (N.A.) (40)	8632 (B.A.) (52)	1·85 (N.A.) (0·049)	38·6 (B.A.) (0·239)

Percentage survival of Organisms: N.A. = Nutrient Agar. B.A. = Blood Agar.

DIPHThERIA PROJECT

Diphtheria project was based mainly on three aspects—

- (1) Biochemical.
- (2) Epidemiological and
- (3) Immunological.

Biochemical studies dealt with the investigation of the role of cellular of different strains of *C. diphtheriae* circulating in this part of the

Earlier studies dealt with the analysis of proteins, R.N.A., D.N.A. and fatty components of Gravis, Intermedius, Mitis and PW-8. It was noted fatty acid composition differed considerably among different strains some relationship between toxin production and fatty acid

The biochemical study was further extended to nucleotide composition of nucleic acids in four strains of *C. diphtheriae*, viz. : Wg-5, Gravis, Intermedius and Mitis. This study was undertaken to explore the possibility of nucleic acid specificity of different strains of *C. diphtheriae*. The base composition of D.N.A. and R.N.A. from four strains of diphtheria is given in the table 1.

TABLE 1

Base composition of DNA and RNA from four strains of diphtheria

Strain type		Base proportion mole percentage					A — T	A + U
		A	T	U	G	G	G + C	G + C
WG-5	{ DNA	.. 26.1	25.9		23.4	24.6	1.08	
	{ RNA	.. 20.2		24.3	29.3	26.1		0.81
Gravis	{ DNA	.. 25.4	26.1		24.7	23.8	1.06	
	{ RNA	.. 21.6		21.0	30.5	27.2		0.75
Mitis	{ DNA	.. 21.5	20.3		29.5	28.4	0.72	
	{ RNA	.. 18.4		22.4	28.2	31.0		0.66
Intermedius	{ DNA	.. 22.6	23.0		27.2	27.3	0.83	
	{ RNA	.. 22.6		22.1	17.1	38.2		1.00

Besides chemical constituents, enzyme study was also undertaken. Since transaminases are key enzymes in the protein synthesis, it was decided to find out the activity of these enzymes. Earlier studies revealed higher levels of oxalacetic transaminase activity in Gravis and PW-8 strains which are known to be toxigenic.

The study was extended to investigate NAD dependent dehydrogenase systems in WG-5, Gravis, Intermedius and Mitis strains of *C. diphtheriae*. Preliminary data on the crude extracts of these organisms is given in table 2.

TABLE 2

Specific activity in the crude extract

Strain			Dehydrogenases				
			Idditiol	Malate	Succinate	Lactate	Alcohol
WG-5	..	..	0.22	0.83	0.65	0.43	0.18
Gravis	..	..	0.26	0.65	0.80	0.51	0.14
Mitis	..	..	0.15	0.95	0.92	0.72	0.31
Intermedits	..	..	0.20	0.63	0.70	0.40	0.28

Studies on confirmation and extension of these data to other various conditions are being continued.

data

Phage typing of locally isolated strains of *C. diphtheriae* was undertaken, a set of 24 phages obtained from W.H.O. Reference Centre in Fifty out of 80 strains belonged to phage type 19.

Study

(a) *Synthetic medium*.—The study of toxin production by *C. diphtheriae* is to obtain a high yield of toxin for the purpose of antitoxin production. Attention is focussed to formulate a highly efficient medium for the production of toxin. A synthetic medium, consisting of 5 amino 5 vitamins, inorganic salts and maltose, has been formulated, which give 75 to 80 Lf/ml. of toxin using *C. diphtheriae* PW-8 strain. Since is the most important constituent in the medium for toxin production, effect of different concentrations of iron was investigated. Iron in 1 mg concentration decreased the toxin production to less than 5 Lf/ml. was also found out that this inhibitory effect of iron could be inhibited by of EDTA or excess of calcium.

(b) *Role of phage in induction of toxin*.—Since lysogenic conversion the production of toxin, it was thought worthwhile to study the of toxin by infecting various strains of *C. diphtheriae* with different

phages. Thirteen phages were obtained from Roumania and one (P) from America. These fourteen phages could be classified into two groups, depending on host specificity. Phages P, 1, 2, 3, 4 and 5 could infect diphtheria strains 1180 and C-7 and phages 10, 11, 12, 14, 15, 16, 18 and 24 could infect diphtheria strains 304 and 3148. It was noted that super-infection of PW-8 strain by phage 3 did not reduce its ability to produce toxin. Phages in recombination were found to exhibit different host range from their parent strains. It was possible to convert non-toxinogenic phage 15 into toxinogenic phage after passing through toxin-producing host strain 3148. Thus, non-toxinogenic strains of *C. diphtheriae* can be made toxinogenic by exposure to appropriate phages.

It has been shown that the specific nucleotide bases involved in this process of lysogenic conversion come from the native D.N.A. of the bacterial cell. This proves the importance of investigating the role of nucleotide base composition of D.N.A. of different toxinogenic and non-toxinogenic strains of *C. diphtheriae*.

Diphtheria toxin production in synthetic medium

A synthetic medium containing 3 amino acids, 5 vitamins, inorganic salts and maltose was formulated (Ref. Immunology Department for details). It was found that *C. diphtheriae* strain PW-8-W, grown on this medium, gave toxin of about 80 Lf/ml. Iron (0.1 mg of FeCl_3) inhibited toxin production. However, inhibition effect of iron could be reversed by adding excess calcium or by EDTA.

Role of phage in induction of toxin

Thirteen phages, received from W.H.O. International Reference Centre in Roumania and one phage obtained from America, were used to study induction of toxin by phage. One group of phages could infect strains 1180 and C₇ while the second group could infect diphtheria strains 304 and 3148. Diphtheria strain, *C. ulcerans* 603, was used as an indicator to detect the phage present in PW-8 strains. Interestingly, Phage 3 could infect PW-8 strain without reducing its ability to produce toxin.

PLAGUE PROJECT

Despite its absence for the last 20 years in the State of Maharashtra, plague continues to pose a threat of recrudescence in view of its probable persistence in the neighbouring States and possibility of presence of foci of infection commensal and wild rodents.

Plague project studies were, therefore, initiated in the State of Maharashtra to detect lingering foci of plague infection, if present, and to undertake surveillance measures against recrudescence. The studies were initiated in the year 1965 with the following objects :—

(1) to ascertain the broad pattern of rat fauna and their specific ectoparasites in the State of Maharashtra ;

(2) to find out if there is any *P. pestis* infection in rats collected from different parts of the Maharashtra State or the presence of antibodies against *P. pestis*, to assess the susceptibility of individual species to rats in different areas, and the type of immunity (tissue or humoral) prevalent in the infected rats.

The work carried out indicated heavy rodent infestation throughout the Maharashtra State wherever the studies were undertaken. The flea index was also high. Commensal *Rattus rattus* showed high degree of resistance to *P. pestis* in urban areas and high degree of susceptibility in hinterland areas where plague was not endemic. Serological studies indicated the likelihood of presence of lingering foci amongst rodents in Mahad and Marathwada region where plague was highly endemic.

In view of these findings, intensive serological studies were contemplated by dividing the State into eight ecologically distinct zones. Unfortunately, these could not be followed up due to economic stringency and studies were restricted to the Municipal Corporation area of Greater Bombay.

During the period under report, these studies were undertaken in 'A', 'D' and 'E' ward areas of Bombay city. *R. rattus*, *R. norvegicus* and *B. bengalensis* together were observed to constitute the predominant commensal rodent fauna. Of these three principal species, *B. bengalensis* was found to be the most predominant.

In all, 2470 rats were examined from 'A', 'D' and 'E' ward areas. *X. cheopis* was predominant in 'D' and 'E' ward areas and in 'A' ward. *X. astia* showed its preponderance over *X. cheopis*. The studies in 'D' and 'E' ward areas are in progress.

Susceptibility status of oriental rat flea to different insecticides

Assessment of susceptibility status of oriental rat flea to D.D.T. and other insecticides revealed a high degree of resistance among fleas to D.D.T. The fleas also showed some tolerance to malathion and fenthion. It was interesting to note that fenthion was never used in Nasik area for insecticidal purposes.

Laboratory evaluation of anticoagulant rodenticides

Periodic assessment of susceptibility status of communal rodents, viz. *R. rattus*, *R. norvegicus* and *B. bengalensis*, collected from different wards of Bombay city, had indicated the existence of anticoagulant resistant strains of *R. rattus*. Therefore, rodents from different wards were examined for assessing their susceptibility to anticoagulant rodenticides. The studies carried out so far revealed the possibility of presence of tolerance amongst *B. bengalensis* to anticoagulant rodenticides in the 'D' ward area.

Investigation in susceptibility of rodent population in Maharashtra to *P. pestis* and related serological studies.

Susceptibility of rats from different areas to plague infection was studied with a standard challenge.

Approximately equal number of rats (male and female) from different areas were taken. They were challenged with a fixed dose of about 5,000 organisms of the virulent *P. pestis* strain 195/P as before and the susceptibility was determined. The passive haemagglutination test was used for testing rodent sera for the presence of the antibody. Rats belonging to the species other than *R. rattus*, viz., *Tatera indica*, *R. blandfordi*, *Golunda SP.* and *B. bengalensis* were collected and their susceptibility status was also determined. The results are presented in the accompanying tables. The rats from the vicinity of Ellora in Aurangabad district showed a uniformly high resistance, varying from 56 to 70 per cent., whereas rats collected from places near Ajanta showed a resistance, varying from 38.4 per cent. to 50 per cent. (See Table 1).

The rats belonging to the species *Golunda ellioti*, *R. blandfordi* and *Tatera indica* showed high susceptibility to *P. pestis* (See Table 2). The rats (*B. bengalensis*) collected from the 'D' ward of Bombay Municipal Corporation showed a resistance of 15 per cent., indicating that the resistance of *B. bengalensis* has not significantly altered (See Table 3).

The *Rattus rattus* collected from the Satara district showed high resistance to plague, viz. 70 per cent. (See Table 4).

The sera of rats collected from Aurangabad and Satara districts were also tested by the haemagglutination test. All the sera showed a titre of less than 1 : 5 indicating thereby the absence of any antibody to Fraction I (See Tables 5 and 6 respectively). This means that there is no evidence of any latent infection in these rats and the resistance of these rats might be due to genetic factors.

Susceptibility of Rats (R. rattus) from Aurangabad district to P. pestis (Strain 195/P)

Place of collection	Date of inoculation	Number of rats inoculated			Number of rats died within 15 days			Number of rats found positive to plague			Percentage of Susceptibility	Percentage of Resistance	
		Male	Female	Total	Male	Female	Total	Male	Female	Total			
Ellora													
Khuldabad	.. 4th December 1970 ..	24	26	50	14	8	22	13	7	20	41.7	58.3	
Mhaismal	.. 4th December 1970 ..	14	16	30	7	7	14	7	7	14	46.7	53.3	
Kagzipura	.. 4th December 1970 ..	10	30	40	5	14	19	5	13	18	46.2	53.8	
Takli ..	.. 4th December 1970 ..	24	26	50	10	12	22	10	12	22	44	56	
Daulatabad	.. 30th April 1971 ..	4	6	10	2	1	3	2	1	3	30	70	
Ajanta													
Feradpur-Ajanta	.. 30th April 1971 ..	28	32	60	17	20	37	17	20	37	61.7	38.3	
Jangali Tanda	.. 30th April 1971 ..	14	16	30	7	8	15	7	8	15	50	50	
Thanwarkhadi	.. 30th April 1971 ..	22	28	50	11	18	29	11	18	29	58	42	

TABLE 2

Susceptibility of rats belonging to different species from Aurangabad district to P. pestis (Strain 195/P)

Species of rats	Date of inoculation	Number of rats inoculated			Number of rats died within 15 days			Number of rats found positive to plague			Percentage of Susceptibility	Percentage of Resistance
		Male	Female	Total	Male	Female	Total	Male	Female	Total		
<i>Golunda ellioti</i>	.. 16th March 1971	.. 12	18	30	12	18	30	12	17	29	100	..
<i>R. blandfordi</i>	.. 16th March 1971	.. 26	22	48	26	21	47	26	21	47	97·9	2·1
<i>Tatera indica</i>	.. 16th March 1971	.. 42	58	100	41	58	99	41	58	99	99	1·0
<i>Rattus rattus</i>	.. 16th March 1971	.. 10	12	22	6	7	13	6	7	13	59·09	40·91

TABLE 3

Susceptibility of rats (B. bengalensis) from Bombay ('D' Ward) to P. pestis (Strain 195/P)

Place of collection	Date of inoculation	Number of rats inoculated			Number of rats died within 15 days			Number of rats found positive to plague			Percentage of Susceptibility	Percentage of Resistance
		Male	Female	Total	Male	Female	Total	Male	Female	Total		
Bombay ('D' ward)	.. 10th August 1971	.. 30	70	100	25	60	85	23	60	83	84·7	15·3

TABLE 4

Susceptibility of rats (R. rattus) from Satara district to P. pestis (Strain 195/P)

Place of collection	Date of inoculation	Number of rats inoculated			Number of rats died within 15 days			Number of rats found positive to plague			Percentage of Susceptibility	Percentage of Resistance
		Male	Female	Total	Male	Female	Total	Male	Female	Total		
Satara ..	.. 30th April 1971	.. 24	26	50	7	8	15	7	8	15	30	70

TABLE 5

Hæmagglutination titre of rat sera (R. rattus) from different places

Place	Species used	Serum Pools used	Pools showing titre		
			1/5	1/5—1/10	1/10—1/640

<i>Aurangabad District</i>							
Mhaismal ..	..	<i>R. rattus</i>	..	7	7	—	—
Khuldabad	..	Do.	..	20	20	—	—
Takli	..	Do.	..	6	6	—	—
<i>Satara District</i>							
Satara ..	..	<i>R. rattus</i>	..	14	12	—	—
Standard ..	..	Do.	..	—	+	+	+ 1/640

TABLE 6

Hæmagglutination titre of rat sera of different species from Aurangabad

Place	Species used	Serum pools used	Pools showing titre		
			1/5	1/5—1/10	1/10—1/640
Aurangabad ..	.. <i>R. Blanfordi</i>	.. 10	10	—	—
Aurangabad ..	.. <i>Golunda ellioti</i>	.. 8	8	—	—
Aurangabad ..	.. <i>Tatera indica</i>	.. 6	6	—	—
Standard ..		.. —	+	+	+ 1/640

B. DEPARTMENTAL RESEARCH

DEPARTMENT OF ANALYTICAL AND CONTROL

Stability of Vitamins in light

Investigation on this problem was continued. The study covered the photolytic effect on different vitamins with particular interest in different regions of the spectrum of light using different dyes and combinations of dyes as filters and time-exposure factor. Vitamins individually and in different combinations and concentrations at varied pH-values are being studied. Data on studies on riboflavin, niacinamide, nicotinic acid and Vitamin C were concisely presented in the last report.

During the period under report, the results revealed that folic acid at pH 9.5 gets destroyed in ultraviolet light and blue regions. No destruction is observed in green, yellow, orange and red regions. Butylated hydroxy-toluene (BHT), Butylated hydroxyanisole (BHA) and ascorbic acid have stabilizing effect on pteroyl glutamic acid (PGA), while in the case of sorbitol, benzyl alcohol and phenol it is not so. Same chemicals were studied at pH 7.0 and same effect was observed. Riboflavin was destroyed at pH 5.8 in the presence of BHA, BHT and BHA + BHT. It was slightly stabilized by BHA. Riboflavin at pH 7.0 was not stabilized by sorbitol, ascorbic acid and phenol but was stabilized to a limited degree by benzyl alcohol. Vitamin B₂ is highly destructive to PGA, but PGA is not so destructive to Riboflavin. BHA and ascorbic acid exert a stabilizing effect on PGA, while others do not. Calcium pantothenate, at different pH levels, is stable in sun light. Vitamin B₆ is destroyed by sun light, its destructive effect being lesser in acidic medium than that in alkaline. Further study is in progress.

Standardization of cholera vaccine

Investigation is afoot to evolve a method for standardization of cholera vaccine by the passive immunisation technique. Hyperimmune sera were prepared in adult rabbits by multiple intravenous dose method. The antibody and vibriocidal titres of individual serum were determined. Different groups of infant rabbits were administered sera in 1 : 3 dilution, 1 : 9 dilution and original and one group of animals was kept as control (unprotected); pre-operation route was intramuscular and post-operation route was intraperitoneal. The intrainestinal challenge to infant rabbit was by live virulent *V. cholerae* Inaba and Ogawa separately. A record of time the cholera symptoms set in, deaths, recovery, absence of symptoms and survivals in each group of study, was maintained. The data was statistically analysed. The work is in progress.

Studies on bacterial flora of vitamin tablets

Cultural study was carried out to isolate and identify bacterial flora from vitamin preparations and other tablets. The most predominant micro-organism encountered was *Staphylococci*, but the aureus type is coagulase negative. Further study is in progress.

Studies on *Vibrio parahaemolyticus* in fish in the coast of Bombay

This marine bacterium gives rise to food-poisoning. Samples from Worli and Mahim coastal regions were culturally investigated and 12 strains of *V. parahaemolyticus* were isolated and morphological, biochemical, physiological, serological and pathogenicity studies were carried out. Gradually, fish samples from the wholly Bombay coast will be studied. Stool samples from cases of gastro-enteritis will be studied and serological survey of fisher-folk will be made. When the study is complete, it will throw some light on the gastro-enteritis of unknown etiology in Bombay.

Synthesis and Screening of organic compounds

A number of derivatives of 2-acylamino-5 thiocyanothiazoles are being synthesized and screened for antibacterial and fungicidal activity.

DEPARTMENT OF BACTERIOLOGY

Development of submerged and continuous culture methods for the growth of micro-organisms

A substantial increase in the growth of *S. typhi* in a twin unit Emenvee fermentor, using casein hydrolysate medium fortified with yeast extract, Sodium P-glycerophosphate and glycerol, was reported last year. It was also mentioned that the production of Vi and H antigens in the fermentor cultures was unsatisfactory and more detailed studies were necessary for working out the optimum conditions for the growth and antigen production.

In order to increase the yield of H and Vi antigens in a culture medium various environmental factors were changed. It was found that neither a change in agitation rate nor a change in the aeration rate improved the production of these antigens. Hence, it was considered that the poor yield of H and Vi antigens might be due to the highly alkaline pH of the culture attained in a short interval of cultivation. To prevent the pH being shifted to the alkaline side, sodium dihydrogen phosphate (0.5 per cent) was incorporated in the medium. This improved the growth of the organisms (about 15 times that of the stationary cultures) but failed to improve the yield of H and Vi antigens. It could also not control the pH of the medium

at 7.4. In another experiment, 0.5 per cent. Glucose and 0.25 per cent. —0.5 per cent. NaH_2PO_4 were incorporated in the medium and the initial pH of the medium was kept at 7.2. It was found that this change further improved the growth of the organism but not the yield of H and Vi antigens. The pH of the medium remained acidic, i.e. 6.4 only up to 14-16 hours of cultivation and then immediately went above 8.0 after 17-18 hours. Addition of extra glucose and NaH_2PO_4 (0.5 per cent. each) to the 17-18 hours' old culture, having pH above 8.0, could neither bring the pH down nor improve the production of H and Vi antigens. It did not show any increase in growth also.

Since the addition of extra glucose and NaH_2PO_4 failed to control the pH, it was decided to check the pH from the beginning and not allow it to go beyond 7.0—7.2. For studying the effect of this change, 0.5 per cent. glucose and 0.5 per cent. NaH_2PO_4 were added to the culture as soon as it attained the pH of 7.0 and the organisms were allowed to grow. Addition of these substances lowered the pH to the acidic side for some time only and slightly improved the production of H and Vi antigens. The growth remained unaffected.

Further attempts are being made to improve the yield of H and Vi antigens by incorporating other chemicals and also by maintaining the pH of the culture medium.

Apart from studying the effect of various factors on the growth and antigenicity of *S. typhi* (Ty-2 strain) in the fermentor cultures, an attempt was also being made to prepare various types of vaccines, viz. formalised, phenolised, alcoholised and acetone-killed and dried vaccines from these cultures. The efficacy of these vaccines will be compared with similar types of vaccines prepared from the cultures grown on routine solid media.

In an attempt to prepare acetone-killed and dried vaccine from the cultures, it was not possible to separate out the cell mass by addition of acetone to the fermentor culture, because the constituents of the culture medium interfered with the separation of the cells. Therefore it was necessary to centrifuge the fermentor culture and free the cells from the interfering constituents by repeated washing of with buffered saline. These cells were further treated by the usual for the preparation of acetone-killed and dried vaccine. Preparation of this vaccine is in progress.

of bacterial cultures

As reported earlier, this work was undertaken to utilize the method of for the preparation of live plague and cholera vaccines. of various factors associated with lyophilization, viz. suspending

medium initial cell-concentration and effect of freezing on survival rate of the three strains, viz. (i) *Pasteurella pestis* (Ev Madagascar), (ii) *Vibrio cholerae* (El tor water 6) and (iii) *Vibrio cholerae* (C₁-S₂), was continued.

In addition to the abovementioned factors, the following factors were also studied for the *Pasteurella pestis* Ev Madagascar strain, viz. (i) effect of slow drying in the menstruum containing mixture of 6 per cent. sodium glutamate and 6 per cent. lactose. (This menstruum had given the highest percentage of survival rate), (ii) survival rate of this strain in plugs prepared with (a) 6 per cent. lactose solution, (b) 12 per cent. lactose solution, (c) 6 per cent. starch solution, (d) 6 per cent. lactose + 6 per cent. sodium glutamate, (e) 20 per cent. lactose and 1 per cent. sodium glutamate, (f) 10 per cent. lactose and 3 per cent. sodium glutamate.

The abovementioned solutions were lyophilized and plugs were prepared. A quantity of 0.1 ml. of culture was dropped on these freeze-dried plugs. The culture was then dried on the Cryochem apparatus. It was observed that the culture did not get absorbed easily. The plugs containing lactose and sodium glutamate mixture did not dry properly.

Virulence test of all the three strains was carried out in order to see if lyophilization brings about any change in this property. Agglutination test was carried out to see if there is any change in antigenic property of organisms after lyophilization. For El tor strain, haemolysin test was also carried out.

The survival rate of the three strains with 6 per cent. lactose and 6 per cent. sodium glutamate as suspending medium with different initial cell-concentrations is given in Table 1, which also shows the survival rate of *P. pestis* Ev Madagascar in 6 per cent. Dextrin.

The survival rate after slow drying is shown in Table 2.

The survival rate in plugs of different substances is shown in Table 3. The percentage survival in case of *P. pestis* varied between 0.14 per cent. to 6.8 per cent. with the mixture of 6 per cent. sodium glutamate and 6 per cent. lactose mixture. With 6 per cent. Dextrin, the survival was poor. A survival rate of 0.008 per cent. to 0.38 per cent. was obtained. After freezing only, the percentage survival in case of the former menstruum was approximately 60 per cent. and in the latter, it varied between 6 per cent. and 26 per cent. No improvement was observed in the survival rate in comparison with the previous experiments.

In case of vibrio strain, the survival rate varied from 0.01 per cent. to 0.4 per cent. When the organisms were reconstituted on enriched medium-like blood agar, the survival rate was three times more than that obtained on nutrient agar.

Slow drying showed decrease in percentage survival. Therefore, use of slow drying was not found advantageous for obtaining higher survival rate. The method of drying the culture on plugs of various menstrua showed a percentage survival carrying between 0.2 and 1.3 per cent. But, this method can be used only for preservation purposes, because the number of initial organisms is small and, therefore, the number of organisms surviving after lyophilization is so poor as not to be of any use for the preparation of live vaccines.

Virulence and antigenic property remain unaltered after lyophilization. This shows that the method adopted for lyophilization has no adverse effect on these two properties of the organisms.

TABLE 1

Percentage survival of P. pestis and V. cholerae strains after freezing and lyophilization

Organisms	Suspending medium	Number of organisms/ml before processing		Number of organisms/ml after freezing only		Number of organisms/ml after lyophilization	
		X 10 ⁶		X 10 ⁶		X 10 ⁶	
<i>P. pestis</i> Ev Madagascar	.. 6 per cent sodium- glutamate + 6 per cent lactose	21,250		11,700		1,450	
				*(55.0)		*(6.83)	
<i>P. pestis</i> Ev Madagascar	.. 6 per cent Dextrin ..	3,275		2,200		4.7	
				*(66.71)		(0.1439)	
<i>P. pestis</i> Ev Madagascar	.. 6 per cent Dextrin ..	53,500		3,346		4.2	
				(6.25)		(0.0079)	
<i>V. cholerae</i> El tor water 6	.. 6 per cent lactose + 6 per cent sodium glutamate	36,000		9,400		137	
				(26.11)		(0.3806)	
<i>V. cholerae</i> El tor water 6	.. 6 per cent lactose + 6 per cent sodium glutamate	65,000	89,500	4,050	10,600	12	12
		(N.A.)†	(B.A.)‡	(N.A.)†	(B.A.)‡	(N.A.)†	(B.A.)‡
<i>V. cholerae</i> Ogawa C ₁₄ — S ₃	.. 6 per cent lactose + 6 per cent sodium glutamate	8,150	11,100	3,000	5,250	16	41.6
		(N.A.)†	(N.A.)†	(N.A.)†	(B.A.)‡	(N.A.)†	(B.A.)‡
<i>V. cholerae</i> Ogawa C ₁₄ — S ₃	.. 6 per cent lactose + 6 per cent sodium glutamate	27,000	77,500	1,566	8,050	65	58.5
		(N.A.)†	(B.A.)‡	(N.A.)†	(B.A.)‡	(N.A.)†	(B.A.)‡
<i>V. cholerae</i> Ogawa C ₁₄ — S ₃	.. 6 per cent lactose + 6 per cent sodium glutamate	4,525	16,600	1,810	8,632	1.85	38.6
		(N.A.)†	(B.A.)‡	(N.A.)†	(B.A.)‡	(N.A.)†	(B.A.)‡

* = Percentage survival of organisms.

†N.A. = Nutrient agar.

‡B.A. = Blood Agar.

TABLE 2

Percentage survival of P. pestis after slow drying

Organisms	Suspending medium	Number of organisms/ml before processing X 10 ⁶	Number of organisms/ml after freezing only X 10 ⁶	Number of organisms/ml after lyophiliza- tion X 10 ⁶
<i>P. pestis</i> Ev Madagascar (slow drying)	6 per cent lactose + 6 per cent sodium glutamate	33163 18163	19,630 (59·2) 9,335 (51·4)	38·5 (0·12) 4·35 (0·024)

TABLE 3

Percentage survival of P. pestis in plugs of different menstrua

Plug made up of	Number of organisms/ml (dropped on plug) X 10 ⁶	Number of organisms/ml after drying X 10 ⁶
6 per cent lactose	1545	6·35 (0·41)
12 per cent lactose	1545	7·1 (0·46)
6 per cent starch	1545	0·44 (0·029)
20 per cent lactose + 1 per cent sodium glutamate ..		
10 per cent lactose + 1 per cent sodium glutamate ..	1545	0·9 (0·058)
6 per cent lactose + 6 per cent sodium glutamate ..	1545	1·93 (0·13)

Radiation-killed cholera vaccine

The investigations were continued with a view to studying whether the use of radiation for killing the cholera organisms would yield a more potent cholera vaccine. It was reported last year that the two vaccines, prepared by irradiating the suspensions of the strain *Vibrio cholerae* 569 B Inaba for one hour and two hours respectively, were much more potent than the usual formalin-killed and phenyl mercuric nitrate preserved vaccine.

During the period under review, two vaccines were prepared by exposing suspensions containing 60×10^9 organisms per ml. of the strain *Vibrio cholerae* S/II/58 Ogawa to a ^{60}Co source for one hour and two hours respectively. These suspensions were then diluted to contain 6×10^8 organisms per ml. with phosphate buffered saline and preserved by the addition of 0.0015 per cent. phenyl mercuric nitrate. The two vaccines were then tested for their antigenicity by the active mouse protection test and compared with a formalin-killed and P.M.N. preserved vaccine prepared from the same strain and containing the same number of organisms per ml.

In another set of experiments, divalent cholera vaccines were prepared by mixing together the suspensions of the strains 569 B Inaba and S/II/58 Ogawa irradiated for one hour and two hours respectively. The mixed suspensions were then diluted to contain 6×10^8 organisms per ml. of each of the two strains and preserved by the addition of 0.0015 per cent. phenyl mercuric nitrate. The vaccines were then tested by the active mouse protection test along with a standard cholera vaccine containing the same number of organisms per ml. of the same strains, but killed by the addition of formalin and preserved by phenyl mercuric nitrate. The results are given below along with one test (Test No. 1) carried out during the previous year :—

Test No.	Vaccine	Challenge strain	ED ₅₀	Relative potency
I	569 B Inaba irradiated for one hour.	569 B Inaba ..	Less than 0.00004	More than 2.75
	569 B Inaba irradiated for two hours.	569 B Inaba ..	Less than 0.00004	More than 2.75
	569 B Inaba formalin-killed	.. 569 B Inaba ..	0.00011	1
II	S/II/58 Ogawa irradiated for one hour	5321 Ogawa ..	0.0000509	2.8
	S/II/58 Ogawa irradiated for two hours	5321 Ogawa ..	0.00009	1.6
	S/II/58 Ogawa formalin-killed	.. 5321 Ogawa ..	0.000145	1

Test No.	Vaccine	Challango strain	ED ₅₀	Relative potency
III	569 B Inaba + S/II/58 Ogawa irradiated for one hour.	569 B Inaba	0.00003	2
	Standard cholera vaccine, (formalin-killed).	569 B Inaba ..	0.00006	1
IV	569 B Inaba + S/II/58 Ogawa irradiated for one hour.	5321 Ogawa ..	0.00002387	2.97
	569 B Inaba + S/II/58 Ogawa for two hours.	5321 Ogawa ..	0.00004395	1.6
	Standard cholera vaccine	.. 5321 Ogawa ..	0.000071	1
V	569 B Inaba + S/II/58 Ogawa irradiated for one hour.	569 B Inaba ..	0.00003085	2.97
	569 B Inaba + S/II/58 Ogawa irradiated for two hours.	569 B Inaba ..	0.0000412	2.17
	Standard cholera vaccine	.. 569 B Inaba ..	0.0000894	1
VI	569 B Inaba + S/II/58 Ogawa irradiated for one hour.	5321 Ogawa ..	0.00005	1.34
	569 B Inaba + S/II/58 Ogawa irradiated for two hours.	5321 Ogawa ..	0.000017	3.9
	Standard cholera vaccine	.. 5321 Ogawa ..	0.000067	1

It will be seen from the above results that the vaccine prepared by irradiating the cultures, either for one hour or two hours, are more potent than the usual formalin-killed and P.M.N. preserved cholera vaccines. It is proposed to prepare a few more batches of irradiated cholera vaccine and subject them to antigenicity test for confirmation of the results obtained. It is also necessary to find out the optimum dose of radiation required to give a highly potent and the least toxic vaccine.

Preparation of allergenic extracts from the pollen of *Parthenium hystrophorous* L

It has been noted that a particular type of grass, namely *Parthenium hystrophorous* L, is growing wildy in certain parts of Maharashtra and the pollens of the same cause allergic reactions, leading to skin ailments like eczema. Studies were undertaken with a view to seeing if an allergin from this particular variety of grass could be prepared and used for sensitizing persons or patients suffering from this allergic disease.

Nine different batches of allergenic extracts of *Parthenium phorous L* (popularly known as "Congress Grass") were prepared. were tested for their sterility after seitz-filtration. Four, out of nine, also been subjected to "Freedom from abnormal toxicity" test laboratory animals. The details regarding the preparation, sterility and toxicity studies are briefly described below :—

Procedure for preparing allergenic extracts—

Defatting

Pollens from the plant were separated and defatted with three solvents namely, ether, acetone and hexane. Defatting was carried out mixing about 100 gms. of pollen with 700 ml. of the defatting. The mixture was effectively shaken at room temperature with portion of defatting agent and the oily solvent extract was then before adding a fresh portion of the defatting agent. The procedure was continued for 48 hours and then the mixture was filtered. The defatted pollens left behind were completely dried at 37°C.

Extraction

For extracting the active principle, dried defatted pollen were treated with three different menstrua, viz.—

(i) *Buffered saline* (used at University of Michigan Hospital) :

Sodium chloride, A. R.	...	...	5.0 gms.
Monobasic potassium phosphate, A. R.	...	...	0.36 "
Dibasic sodium phosphate, A. R.	...	...	7.0 "
Phenol crystals, A. R.	...	...	4.0 "
Water for injection to adjust the	...	...	1000.0 ml.
final pH	...	...	8.0

(ii) *Coca's solution* :

Sodium chloride, A. R.	...	...	20.0 gms.
Phenol crystals, A. R.	...	...	16.0 "
Sodium bicarbonate, A. R.	...	...	10.0 "
Water for injection	...	...	4000.0 ml.

(iii) *Glycerinated Coca's solution* :

Sodium chloride, A. R.	...	...	2.5 gms.
Phenol, A. R.	...	...	2.0 "
Sodium bicarbonate, A. R.	...	...	1.25 "
Glycerine, A. R.	...	...	500.0 m.
Water for injection	...	...	1000.0 ml.

Extraction was carried out by soaking dried pollen (already defatted) for 27-72 hours at room temperature with occasional shaking. For arresting the bacterial fermentation, 2.0 ml. of toluene was also added to the mixture.

Clarification and sterilization

After extraction of the material, the solvent containing active principle was primarily coarse-filtered through muslin cloth and then centrifuged at 10,000 r.p.m. for about 30 minutes. The supernatant thus obtained was then sterilized either by Millipore or Seitz-filtration technique. Thus, by using three defatting agents and three different extracting solvents, nine different batches of allergenic extracts were prepared which are listed below :—

- (i) Buffered saline extract of pollen defatted with ether.
- (ii) Coca's solution extract of pollen defatted with ether.
- (iii) Glycerinated Coca's solution extract of pollen defatted with ether.
- (iv) Buffered saline extract of pollen defatted with acetone.
- (v) Coca's solution extract of pollen defatted with acetone.
- (vi) Glycerinated Coca's solution extract of pollen defatted with acetone.
- (vii) Buffered saline extract of pollen defatted with hexane.
- (viii) Coca's solution extract of pollen defatted with hexane.
- (ix) Glycerinated Coca's solution extract of pollen defatted with hexane.

Sterility Test

After filtration, all the nine batches of allergenic extract were tested for their sterility by transferring about 1.0 ml. of each of the extract in Robertson's cooked meat medium N-broth and Sabouraud's broth aseptically and then incubating the first two media at 37°C and the third one at 25°C for 10 days. All the batches were found to be sterile.

Toxicity Test

Of the nine batches, four were tested for their toxicity in laboratory animals. They were :—

- (i) Buffered saline extract of ether defatted pollen.
- (ii) Coca's solution extract of ether defatted pollen.
- (iii) Buffered saline extract of acetone defatted pollen.
- (iv) Coca's solution extract of acetone defatted pollen.

Toxicity of these batches was tested in mice weighing from 18 to 20 gms as well as in guinea-pigs weighing from 250-350 gms. In both the animals, the route of injection was subcutaneous. Mouse was injected

with 0.5 ml. and the guinea-pig with 5.0 ml. of sterile extract. The animals were then observed upto 7 days for the development of toxic symptoms.

Of the four batches subjected to the toxicity test, batch No. (ii), i.e. Coca's solution extract of ether defatted pollen, was found to be toxic only in the mice, as it caused death within 4 days, whereas the other three batches were found to be non-toxic both in mice and guinea-pigs.

Rest of the batches have yet to be tested for their toxicity in these laboratory animals. In addition to this, antigenic status of these extracts has also to be established.

Storage stability of typhoid vaccine at high altitudes

The studies on the effect of storage of typhoid vaccine under frozen conditions or the effect of repeated freezing and thawing on the protective efficacy of the vaccine, undertaken with the grant-in-aid from the Ministry of Defence (Research and Development Organisation), were continued. The scheme came to an end in December 1971.

It was reported during the previous years that the three types of vaccines, viz. (i) formalin-killed and phenyl mercuric nitrate (P.M.N.) preserved T.A.B. vaccine, (ii) heat-killed and phenol preserved T.A.B. vaccine and (iii) phenol-killed and preserved typhoid vaccine did not show any deterioration in potency as shown by mouse protection test, when stored at -35°C for one year. It was also reported that there was no deterioration in potency of T.A.B. vaccine, either heat-killed and phenol-preserved or formalised and P.M.N. preserved, when subjected to repeated freezing at -35°C in a deep-freeze and thawing six times at 40°C . Further, it was shown that the agglutinin response in rabbits to the vaccine stored at -35°C for 6 months was slightly better than that obtained with the same vaccine stored at 4°C .

During the year under review, the three types of vaccines stored at -35°C for a period of 15 months and 18 months were subjected to active mouse protection test along with a vaccine of the same batch stored at 4°C . Each test was repeated thrice. The results are recorded in table 1.

To study the effect of repeated freezing and thawing of the vaccines subjected to natural conditions at high altitudes, samples of two types of T.A.B. vaccines (i) formalin-killed and P.M.N. preserved and (ii) heat-killed and phenol preserved, were forwarded to the field laboratory. The laboratory was requested to thaw at weekly intervals two of the samples of each type by keeping them in a water bath at 40°C for a time just sufficient to melt them and then to forward them to this Institute. This experiment was extended at the field laboratory upto the sixth thaw

Samples after the first, third and sixth thaw, received from the field laboratory, were then tested for their antigenicity by active mouse protection test along with a sample of the same batch of the vaccine stored at 4°C. The results are presented in table 2.

The antigenicity of the formalin-killed and P.M.N. preserved T.A.B. vaccine, stored at -35°C for 9 months and 18 months and also of that stored at 4°C was studied by immunizing adult rabbits. Three rabbits were used for testing each sample. Each rabbit was injected with two doses of 0.5 ml. and 1.0 ml. of 1/10 dilution of the vaccine intravenously at an interval of 7 days. The rabbits were bled just before the immunization and then 7 days after the first and second dose. The separated sera were tested by agglutination test using Vi, O, H, para A and para B antigens. The results are given in table 3.

None of the three vaccines, viz. (i) formalin-killed and P.M.N. preserved T.A.B., (ii) heat-killed and phenol-preserved T.A.B., (iii) phenol-killed and preserved typhoid vaccine, when tested by active mouse protection test, showed any fall in potency after storage at -35°C for 15 and 18 months respectively (*see* table 1). Vaccine No. (i) did not show any change in physical appearance upto a period of about 15 months at -35°C. However, it started showing some granulation after this period, which was almost complete within the next three months. Vaccine No. (ii) was stable only for a period of about 6 months, after which period, at -35°C, it started showing granulation increased further with the period of storage. The formation of granules was even more prominent in the case of Vaccine No. (iii). In this case, the granulation started after a storage of 3 months only and went on increasing after that period. After a period of one year of storage at -35°C, the vaccine was completely granulated, showing a clear menstrem separated from the granules.

There was no deterioration in potency of T.A.B. vaccines, either formalin-killed and P.M.N.-preserved or heat-killed and phenol-preserved, on repeated freezing and thawing 6 times at the field laboratory at high altitudes (*see* table 2). The physical appearance of both the vaccines also remained unchanged.

The agglutinin response in the adult rabbits (*see* table 3) to formalised vaccine at -35°C also showed that the vaccine did not undergo any in its antigenicity. In fact, the O, H, para A and para B of the vaccine were after preserved, as they produced significantly response as compared to that produced by the same batch of vaccine at 4°C. The results reported earlier have shown that even the antigen is better preserved.

TABLE 1

Potency of T. A. B. vaccines stored at — 35°C and 4°C.

Period of storage	Temperature of storage	Formalin-killed and P.M.N. preserved T.A.B. vaccine		Heat-killed and phenol-preserved T.A.B. vaccine		Phenol-killed and phenol-preserved typhoid vaccine.	
		Mean ED ₅₀ *	Relative potency**	Mean ED ₅₀ *	Relative potency**	Mean ED ₅₀ *	Relative potency**
15 months	— 35°C	0·0076	1·59	0·00928	1·46	0·00615	1·19
	4°C	0·0116		0·01354		0·00729	
18 months	— 35°C	0·0123	1·0	0·0063	1·19	0·00527	0·99
	4°C	0·0123		0·00751		0·00522	
* Mean of 3 tests.		** Relative potency =		ED ₅₀ of vaccine stored at 4°C.			
				ED ₅₀ of vaccine stored at — 35°C.			

TABLE 2

Potency of T. A. B. vaccines subjected to repeated freezing and thawing at the field laboratory

Serial No. of thaw	Formalin-killed and P.M.N. preserved T.A.B. vaccine.			Heat-killed and phenol-preserved T.A.B. vaccine.		
	Mean ED ₅₀ * of		Relative potency	Mean ED ₅₀ * of		Relative potency
	Frozen and thawed vaccine	Vaccine stored at 4°C		Frozen and thawed vaccine	Vaccine stored at 4°C	
1	0·017	0·0147	0·86	0·00708	0·0089	1·26
2	0·01285	0·01319	1·03	0·00703	0·00835	1·19
3	0·01449	0·0118	0·81	0·00773	0·00803	1·04

TABLE 3

Agglutinin response in rabbits immunized with formalin-killed and P. M. N. preserved T. A. B. vaccine stored at — 35°C and at 4°C.

Temperature of storage and period	*Geometric mean agglutinin titres of the sera														
	Preimmunization					After one dose					After two doses				
	Vi	O	H	para A	para B	Vi	O	H	para A	para B	Vi	O	H	para A	para B
— 35°C 9 months.	..	25	10	10	20	1016	806	1016	126·9		1280	806	508	201·6	
— 35°C 18 months.	..	40	31·75	10		1016	1016	508	80		806	508	806	253·7	
4°C	..	10	18·88	10		640	508	403	80		806	508	640	160	

* Titre is expressed as reciprocal of the geometric mean of the dilution showing positive agglutination.

Geometric mean of three rabbit sera is taken.

DEPARTMENT OF BIOCHEMISTRY

Dietary fats and lipid metabolism

Hypercholesterolemia is considered to be of primary importance in the pathogenesis of atherosclerosis. In many studies, correlation has been observed between—

- (a) fat content of the diet and blood cholesterol levels, and
- (b) blood cholesterol level and incidence of atherosclerosis.

On the basis of these observations, a great many studies (including those carried out in our laboratory), concerning dietary means of altering blood cholesterol levels in animals and/or in human beings, have been reported. It is now established that a correlation exists between the degree of unsaturation of dietary fat and the blood cholesterol level. Recently, a correlation was also observed between sucrose content of the diet and the blood cholesterol levels. In view of these facts, the effect of quality and quantity of dietary fats and carbohydrates on the lipid metabolism was investigated.

The studies on higher level of sucrose in diet, reported last year, were further extended to other sugars, viz.: glucose and lactose (0 to 60 per cent.), potato and corn starch (0 to 60 per cent.) in the presence of the dietary fats, sunflower seed oil and 'Dalda' (10 per cent.). Casein was used as the nitrogen source fed at 24 per cent. level. The feeding experiments, using male albino rats, weighing 140-150 gms. were carried out for a period of ten weeks. The animals were sacrificed at the end of the feeding period for processing the tissues.

The results obtained showed that : (a) as compared to the starches (corn and potato) and glucose, sucrose and lactose in the diet elevated serum and hepatic cholesterol levels, (b) higher levels of sucrose in the diet showed more triglycerides in serum and liver of rats as compared to the starches and lactose, (c) the total liver fat in rats, fed on sucrose, was more than amongst those fed on the starches and lactose.

The results of cholesterol in serum and liver, when compared between the two sets of experiments in respect of feeding 'Dalda' and sunflower seed oil, showed that the 'Dalda' elevated the level of cholesterol in serum and lowered it in liver.

Hormonal regulation of lipid metabolism in relation to dietary carbohydrates

Hormones have been shown to take part in lipid metabolism through cyclic-AMP. Sucrose elevates the level of cholesterol and triglycerides in serum of rats. It was, therefore, thought worthwhile to study the effect of three hormones—adrenaline, thyroxine and insulin on lipid metabolism in rats maintained on two different diets containing sucrose and starch

Groups of male weaning rats were maintained on sucrose/starch, with safflower oil as the dietary fat, for a period of 8 weeks. The hormones were injected in appropriate doses daily for one week, after which the animals were sacrificed for processing of the tissues for cholesterol and phospholipids.

Studies on the nutritional requirements of *V. cholerae*

The study of the growth of the organism (*V. cholerae*) in hydrolysates of isolated proteins, free from carbohydrates and other impurities present in the whole defatted cakes (peanut, sesame, safflower cakes and gawar proteins), was reported last year. While all the other hydrolysates gave fairly good growth for *V. cholerae*, those from safflower seed proteins showed only 50-60 per cent. growth. With a view to elaborate the various nutrient needs of this organism, the hydrolysates were analysed for the minerals—Ca, Mg, Fe, Cu, Na, K, P, chloride and sulphate. Seventeen amino acids were analysed from casein and peanut protein hydrolysates.

Based on the above analyses, a synthetic medium containing glucose, 18 amino acids and mineral salts simulating casein hydrolysate, was used to study the requirements of amino acids and minerals for *V. cholerae* using the standard procedure of omission of a single nutrient at a time. Growth studies were carried out using two strains of *V. cholerae* (*Ogawa S/II/58* and *Inaba 569B*) and two *El-tor* strains (*Ogawa* and *Inaba*). The results indicated that only aspartic acid is absolutely essential for growth, while glycine, histidine and glutamic acid are required for optimum growth and the other amino acids are not essential. Mg, Ca, S and chloride are essential for the growth of all the strains, while Na, Ca, K are required only to give optimum growth. The requirement of calcium could be reduced by increasing the level of Mg.

The following chemically-defined medium has been found to be suitable for the growth of the organism :—

				Millimoles/litre
Sodium chloride	...	...	...	85.55
MgSO ₄ (hydrated)	...	...	...	0.40
Ammonium sulphate	...	...	...	7.57
Disodium hydrogen phosphate	...	...	...	2.87
L-aspartic acid	...	...	...	3.75
L-histidine	...	...	...	1.29
Glycine	...	...	...	6.66
Glutamic acid	...	...	...	2.03
D-glucose	...	...	...	5.55

The growth of cholera vibrios in this synthetic medium was found to be 75-80 per cent. as compared to that of the standard casein hydrolysate (135 mg. N/100 ml.). The synthetic media of Ranta and McLead (1950) and Thind (1961) were used for comparison of the results.

TABLE 1

Growth of V. cholerae in terms of optical density at the end of 72 hours at 37°C.

Medium	Optical density			
	Inaba 569B	Ogawa 5/II/58	El-tor Ogawa	El-tor Inaba
1. Casein hydrolysate (135 mg N/100 ml) ..	0.36	0.38	0.45	0.49
2. Peanut protein hydrolysate (135 mg N/100 ml).	0.45	0.33	0.48	0.54
3. Synthetic medium by Thind (1961) ..	0.23	0.28	0.32	0.35
4. Synthetic medium by Ranta and McLead (1950).	0.24	0.22	0.30	0.32
5. Synthetic medium (formulated) ..	0.27	0.30	0.36	0.40

Biochemical studies on *V. cholerae* (569B Inaba) with reference to the action of furazolidone

Furazolidone (5-nitro-3-amino-2-oxazolidone) is now available commercially for clinical use. It was found to be more effective than chloromycetin and tetracycline in laboratory tests and clinical trials. It was reported last year that concentrations as low as 150-200 μ g of this drug in a synthetic medium inhibit the growth of the two strains of *V. cholerae* by increasing the lag period. *V. cholerae* organisms were found to be more sensitive to the drug than the *El-tor* organisms. The estimations of the cellular constituents indicated that the drug hampered protein synthesis and stimulated RNA synthesis. Major portion of the drug absorbed by the cells is associated with the lipids and nucleic acids.

(a) *Absorption of the drug and leakage of the macromolecular pool.*—Further studies have shown that the maximum amount of the drug is absorbed during the log phase. There is a leakage of free amino acids and nucleotides into the medium and it reaches the maximum by the end of 5 hours.

(b) *Incorporation studies—*

Incorporation of glucose-U-C¹⁴ and glycine-1-C¹⁴.

Labelled nucleotides of Adenine, guanine and uracil.

Since glucose is a common precursor of all the macromolecules in the cell, glucose-U-C¹⁴ was used for comparing the various biosynthetic processes in the normal and drug-treated cells. Glucose is oxidised more and incorporated less by the drug-treated cells. Glycine, a common precursor of proteins and nucleic acids, is incorporated more (about double) in the RNA fraction and less (about half) into the proteins, indicating that the drug acts by inhibiting some steps at translational level of protein biosynthesis.

TABLE 2
Percent incorporation of glucose-U-C¹⁴ and Glycine-1-C¹⁴

		Total incorporation	Saline wash	Acid soluble pool	Lipids	RNA	DNA	Proteins
Glucose	{ Control	29.01	2.60	4.12	11.60	1.84	3.45	5.40
	{ Test ..	24.38	2.45	1.78	8.70	4.40	3.70	3.55
Glycine	{ Control	38.05	5.20	3.30	2.10	3.80	0.85	12.90
	{ Test ..	25.80	5.80	2.00	1.72	8.05	1.26	5.70

TABLE 3
Specific incorporation of radio activity into the macromolecules
(Counts 10³/unit optical density)

			RNA	DNA	Proteins
Glucose	{ Control	..	10.6	71.0	286.0
	{ Test	..	17.0	30.0	73.4
Glycine	{ Control	..	245.0	153.1	429.5
	{ Test	..	479.8	149.2	234.7

Experiments on the kinetics of incorporation of nucleotides indicated there is increased uptake of these by drug-treated cells during six hours compared to the normal.

(c) *Effect of the drug on the RNA sub-fractions.*—Drug-treated cells produced abnormal rise in the synthesis of all sub-fractions of RNA, particularly 16s and 23s. However, the base composition of the RNA sub-fractions was not changed.

(d) *Effect of the drug on transaminase and phosphatase activity of V. cholerae.*—GOT and GPT are catabolic enzymes. The GOT activity is double in the drug-treated cells.

					Control	Test
GOT	..	..	..	..	13.25	26.20
GPT	..	..	..	..	7.50	8.20

Expressed as pyruvic acid units/mg of the cells/hour.

The acid and alkaline phosphatases are not affected by drug treatment.

Biochemical studies on vibrio and *El tor cholerae* with reference to the action of ampicillin, chloromycetin and berberine

There are only few reports on the effectiveness of drugs on human cholera. Chloromycetin and tetracycline have been used with some success in cholera epidemics, while the alkaloid berberine is reported to be quite effective in experimental cholera in young rabbits. The mechanism of action of these drugs is not yet fully understood. In continuation of the earlier studies in this laboratory, work was undertaken to compare the effect of some commonly used drugs on the metabolism of *Vibrio* and its biotype *El-tor cholerae*. Incidentally, this study would throw some light on the fundamental biochemical differences in these two strains of cholerae which are reflected in the severity of the disease caused by them.

For this study, the two strains, *Vibrio* and *El-tor cholerae* and three drugs—(1) ampicillin, (2) chloromycetin, and (3) berberine were chosen.

Ampicillin (alpha-amino benzyl penicillin) was first synthesized by Doyle *et al*, (1961). This antibiotic is shown to be effective in low minimal inhibitory concentrations against a wide variety of gram-negative and gram-positive organisms. It apparently owes its activity and stability to the presence of the free amino group at the alpha position of the N-acetyl side chain of the molecule.

Chloromycetin (p-nitrophenyl-2-dichloro- α -cetamide-1-3-propane) was the first broad spectrum antibiotic to be discovered by Burkholder (1947). It is active against a wide range of gram-negative and gram-positive bacteria, but much less active than penicillin and tetracycline against gram-positive cocci. The structure of the side chain seems to be critical for antibacterial activity.

Berberine is one of the indigenous alkaloids, which has been known from olden days (Gupta, 1908), and has been used for the treatment of cholera and choleraic diarrhoea.

Growth.—The cells of *Vibrio* and *El tor cholerae* were grown in a synthetic medium consisting of amino acids and salts in the presence of increasing concentration of ampicillin (10-200 μ gs./ml.), chloromycetin (0.5-5.0 μ gs./ml.) and berberine (10.0-200.0 μ gs./ml.). Ampicillin, in a concentration of 25 μ gs./ml., gave 50 per cent growth inhibition for both the strains, while chloromycetin produced the same inhibition in as low a concentration as 0.5 μ g./ml. for both the strains. To bring about a similar effect, 25 μ gs./ml. (for *Vibrio*) and 50 μ gs./ml. (for *El-tor*) of berberine are required.

(b) *Cellular constituents.*—The cellular constituents and cell metabolism are effected by changes in the environment in which they are grown. Factors like growth medium, temperature of incubation, stationary and shaken conditions, presence of other substances like antibiotics or drugs, affect the cellular composition. This study includes the effect of the three drugs on the cellular constituents of *V. cholerae* and *El tor cholerae*.

The cellular macromolecules, such as proteins, nucleic acids, carbohydrates and phospholipids, were studied both in the normal and treated cells under shaken condition. All the three drugs were found to change the cellular composition to an appreciable extent. Ampicillin-treated cells showed a decrease in all cellular constituents while chloromycetin-treated cells showed a lower content of proteins and higher percentage of RNA. Berberine-treated cells gave rise to higher content of protein and lipids.

(c) *Glucose metabolism.*—Since glucose gets incorporated into almost all the cellular macromolecules, the metabolic study using labelled glucose can give a clearer idea of the biosynthesis of macromolecules.

The studies were made using uniformly-labelled glucose in the growth medium of normal and drug-treated cells under shaken condition. The cells were fractionated using standard procedures to study the incorporation of glucose into the cellular constituents.

The radioactivity measurements of the macromolecules showed that the ampicillin-treated cells utilize less glucose than the chloromycetin-treated ones in the synthesis of RNA. For lipid fractions, the berberine-treated cells used more sugar.

(d) A study of the isolation and characterisation of the proteins and ribonucleic acids from chloromycetin-treated and untreated cells of *Vibrio* and *El-tor cholerae*, using polyacrylamide gel electrophoresis, is being undertaken.

Haptoglobin levels, phenotype distribution and Hp¹ gene frequencies in normal and pathological cases.

This study was carried out with 123 samples, kindly supplied by Dr. Mirchandani from the J. J. Groups of Hospitals, Bombay and normals from the Blood Bank Department of the Institute.

TABLE 4

Haptoglobin levels, phenotype distribution and Hp¹ gene frequencies in normals and pathological cases

(Hp levels are expressed in mg of bound Hb or its derivative/100 ml).

Group	No. of cases	Mean Hp level in mg. % $\pm$ S.D.	Percentage of Hp phenotypes				Hp ¹ gene frequency
			1-1	2-1	2-2	0-0	
Normals ..	20	104.00 $\pm$ 26.90	5.0	30.0	65.0	0.0	0.20
Infectious hepatitis ..	60	34.36 $\pm$ 28.65	0.0	38.2	25.5	36.3	0.30
Cirrhosis of liver ..	28	57.00 $\pm$ 35.45	0.0	41.5	43.7	14.8	0.24
Malnutrition ..	15	49.20 $\pm$ 37.10	0.0	23.1	46.1	30.8	0.17
Total ..	123						

Protein fractionation on polyacrylamide gel

An elegnat micro technique, using polyacrylamide gel (5 mm. $\times$ 5 cm.) for fractionating micro quantities of proteins in biological fluids has been standardised in the laboratory. The electrophoresis was done at 20°C for 2 hours, using 4 mA current per gel. The gels were stained with amido black and the protein patterns were scanned, using a microdensito meter (by courtesy of the Radiation Medicine Centre, B.A. R.C.) for quantitation. This has been found to be very handy in studying and characterising bacterial, ribosomal, cytoplasmic proteins, and ribonucleic acids in research work.

(a) At the request of the Wadia Children Hospital, some C.S.F. samples [normal (42) and pathological (58)] were screened for protein patterns (qualitative and quantitative). The above technique was found to be useful in the early diagnosis and prognosis of encephalitis polyneuritis, pyogenic and tubercular meningitis.

(b) The same technique was used to screen urine samples of nephritic syndrome cases (22) from the K.E.M. Hospital, Bombay.

(c) Assistance was extended to the Cancer Research Institute to screen milk whey proteins from normal and experimental mice (15 samples) using the above technique.

DEPARTMENT OF CHEMOTHERAPY

Chemotherapy of tuberculosis and leprosy

New terephthalanilides.—Terephthalanilides and terephthalamides have been described as possessing interesting activities including antitubercular, antitumor, etc. In our previous studies, we had found anilines having methyl or dimethyl thiazole as substituents to form potent moieties for antitubercular compounds. Accordingly, we have now synthesised newer terephthalanilides containing these and other related moieties. About a dozen compounds thus prepared were tested for *in vitro* antitubercular activity, but no activity was detected. Since all these compounds are extremely insoluble, their methyltoluene sulphonates are now being prepared for further testing.

Synthesis of thiosemicarbazide and related derivatives

Thiacetazone, clinically used antitubercular drug obtained by reacting p-acetamino-benzaldehyde with thiosemicarbazide, is having unduly high toxicity in human beings, while in laboratory animals, its toxicity is of a much lower order. Presuming this to be due to easier hydrolysis of the azomethine linkage in the human beings liberating the thiosemicarbazide and the aldehyde both of which are known to be highly toxic—we had proposed to use ketones in place of aldehyde to get a stronger bond less amenable to hydrolysis, and had reported in the previous year the synthesis of such compounds having good *in vitro* activity. As a further development of this work, we have now synthesised a series of compounds wherein, instead of the substituted acetophenones, we have used benzylmethylketone and phenethylmethylketone and condensed them with substituted thiosemicarbazides. It has been found that the nature of the substituent on the thiosemicarbazide controls the antitubercular activity. In particular, alkoxyphenyl, viz. : *O*-ethyl and *O*-propyl increased the activity, confirming our earlier observations on the potentiating effect of these substituents.

Hydroxamic acid derivatives

A series of hydroxamic acid derivatives from aryl substituted 1,3,4-oxadiazole-5-mercaptoethylacetate has been synthesised for investigating their antitubercular activity since 5-mercapto-2-(2'-hydroxy-4'-amino) 1,3,4-oxadiazole has been reported to possess good antitubercular activity. These compounds were prepared from 2-aryl-1, 3, 4-oxadiazole-5-mercaptoethylacetate by the action of hydroxylamine hydrochloride in potassium hydroxide. These compounds are at present under testing.

Chemotherapy of protozoan diseases

*N*¹-methyl-*N*⁴-[α -substituted benzylamino] alkyl] piperazines.—In continuation of the earlier work on R-1396 (*N*¹-methyl-*N*⁴[2-*p*-chlorobenzhydrylamino] ethyl piperazine), further variations in the same molecule have been carried out. The length of the bridge between the two nitrogen atoms has been increased from 2 to 3 and also a branching has been effected at the carbon atom linked to the *N*¹-nitrogen of the piperazine ring. Also further variations to change the solubility patterns of these compounds are underway. The study of the *in vitro* activity of all these compounds has shown that the lengthening of the chain has not adversely affected the activity.

1-substituted-2-(*N*⁴-substituted piperazinyl)-vinyl-5-nitro imidazole.—These compounds were prepared as an extension of the earlier work reported last year. Substituted formyl piperazines were condensed with 2-methyl-1-*B*-hydroxyethyl-5-nitro imidazole to obtain the title compounds with a view to see whether this would potentiate the antiamoebic and antitrichomonal activity. These compounds are being studied for their *in vitro* activity.

Synthesis and study of compounds for antitumor and other miscellaneous activities

Schiff bases from substituted 4-p-aminophenyl thiazoles and substituted p-[Bis [2-(methylsulfonyl) oxyethyl (or propyl)] amino] benzaldehydes.—We have earlier reported interesting antitumor activity of certain Schiff bases. For example, the Schiff base from 4-[*N*, *N*'bis (2-chloroethyl) amino]-*m*-anisaldehyde and 4-*p*-aminophenyl-2-phenoxymethylthiazole showed 6/6 cures against Dunning Leukemia at 11 mg./kg. per day, while a Schiff base from the same aldehyde and 4-*p*-amino-phenyl-2-benzyl-5-methyl thiazole showed a good activity against L 1210 lymphoid leukemia and Walker carcinosarcoma 256 (im). The Schiff base from the same aldehyde and 4-*p*-aminophenyl-2, 5-dimethyl thiazole was the most active compound in this series against L 1210 giving 2 out of 6 cures at 25 mg./kg. per day. This compound was also found active against P 388 Lymphocytic leukemia giving 3 cures out of 6 animals treated at 16.5 mg./kg. per day.

Since mustards as a class are more toxic and also because recent work, wherein Cl- of the mustard is replaced by methane sulfonate has shown better tolerance, it was proposed to replace the mustard arm of the above active compounds by methane sulphonate and the Schiff bases from *p*-[Bis [2-(methylsulfonyl) oxyethyl (or propyl)] amino] benzaldehydes and substituted 4-*p*-amino phenyl thiazoles and other amines have been prepared. About thirteen compounds have been characterized and submitted to Cancer Research Institute, Bombay, for cell culture studies against HeLa and KB cells and their results are awaited.

Schiff bases from halogen substituted anilines and substituted p[Bis[2-(methylsulfonyl)oxyethyl (or propyl)]amino]benzaldehydes.—In another series from this laboratory, very active Schiff bases were obtained from 4 [N, N'-bis (2-chloroethyl) amino]-*m*-anisaldehyde and 2, 6-dichloro and 2, 6-dibromo-4-aminophenol as well as *m*-chloroaniline.

These compounds showed high activity against L 1210 lymphoid leukemia and P-388 Lymphocytic leukemia.

This work has now been extended and the "mustard arms" in these active moieties are being replaced by "methane sulfonates".

Pyrimidine and dihydrofuroypyrimidines.—A series of 2, 4-disubstituted-5- β -hydroxy-ethyl (or propyl) pyrimidines has been synthesised for purposes of a broad screening programme. These compounds were also cyclised to obtain dihydrofuroypyrimidines. In all, about 40 new compounds have been prepared. None of the compounds was found to possess any significant antitubercular, antifungal or antiamebic activity (*in vitro*). In view of the fact that pyrimidines are important constituents of nucleic acids, about 30 of these compounds have been sent for screening for antitumor activity to CCNSC (U.S.A.). The cyclised compounds, viz., 5, 6-dihydrofuro (2, 3-d) pyrimidine have also been studied for antifertility activity in collaboration with Department of Pharmacology, in view of the fact that benzofuran and naphthofuran derivatives have been reported to possess oestrogenic activity. In preliminary tests, 6 compounds have shown 60 per cent. antifertility activity in female rats and these compounds are now being studied at other dose levels.

DEPARTMENT OF CLINICAL PATHOLOGY

Studies on gonococcal urethritis

Chacko-Nair's egg medium was used for isolation of *Neisseria gonorrhoeae* from 17 clinically suspected cases of gonorrhoea. *Neisseria gonorrhoeae* were isolated on culture examination from 13 out of these 17 cases. Antibiotic sensitivity studies and analysis of antigenic structure of these local strains will be undertaken.

A new simple composite medium for the identification of Enterobacteriaceae

A new simple composite medium, which can give more information and which has some advantages over the other composite media in common use has been prepared in our laboratory. This new composite medium is divided into 2 parts. Part I indicates fermentation of glucose and lactose, H_2S production and phenyl pyruvic acid test. Part II helps in the detection of

mannitol and sucrose fermentation, motility and indole production. simple and sensitive medium was put to trial with conventional media. was observed that out of 125 strains tested, 122 gave identical results either method.

Tissue culture method for Toxigenicity testing of diphtheriae

Ninety strains of *C. diphtheriae* have been studied for toxigenicity primary monkey kidney tissue culture medium and the results were with the conventional *in vitro* test according to the method of Elek. is in progress.

Standardization of Streptolysin "O" for the determination of O titre.

Streptolysin "O" antigen has been prepared on a large scale. This gen is used for the estimation of Antistreptolysin "O" titre in sera patients suffering from various streptococcal diseases. It is proposed compare the results obtained by the locally prepared streptolysin "O" those of standard imported antigen.

Studies on the Brucellosis in man and animals

A total of 738 blood samples were screened for the evidence of comprising of 73 normal blood samples from prisoners, 52 blood from dairy workers and 613 blood samples from cases of pyrexia unknown origin. The work is in progress.

Use of antigen labelled with radio-isotopes in serological epidemiology

This is a research project under the International Atomic Energy for 1972. Dr. M. V. Sant and Dr. S. Mukerji of the Haffkine Institute and Dr. R. D. Ganatra of the Medical Division of the Bhabha Atomic Centre at the Tata Cancer Research Institute, collaborated with Dr. Felsenfeld of the Tulane Research Centre, Louisiana, U.S.A., to carry study on this problem. At present, *Salmonella typhi* is tagged with Chlorella protein hydrolysate C-14 isotope for radio-immune assay. Work is progress.

Liver damage on feeding rats with alcohol

Animals were divided into two groups. One group was fed with diet and the other with low-protein diet. Control group consisted of animals fed with normal diet and water. Preliminary experiments with 20 per cent. alcohol feeding for 16 weeks did not show any significant changes in the liver. Hence, the concentration of alcohol was increased and the diet was modified. The work is in progress.

Serotyping of *E. coli* in Urinary infection

Preparation of Antisera against 12 common pathogenic serotypes was underway. This will help in finding out the pathogenic serotypes prevalent in this part of the country. Antibiotic sensitivity of all the isolated strains was also carried out.

Fluorescence microscopy in the identification of *M. tuberculosis* in tissue sections

Two hundred eighty tissue sections from suspected tuberculous granulomas were stained by the combination of Auramine and Rhodamine dye for fluorescent microscopy. Positive results were obtained in 80 per cent. of cases. The results indicate that this staining procedure is a valuable adjunct to the histological routine to establish the diagnosis of tuberculous granuloma.

Preparation of indigenous reagents for the detection of R. A. factor in the diagnosis of Rheumatoid arthritis

Latex particles of the size of 0.81 micron were coated with human gamma globulin fraction II in borate buffer. The results obtained by these reagents gave 100 per cent. co-relation with those obtained by the imported Hyland kit. So far, 250 sera have been examined.

DEPARTMENT OF IMMUNO-HAEMATOLOGY

Studies on genetical characters in some endogamous groups in Indian population

Studies on genetical characters comprising of blood groups, viz. (ABO, MNSs, Rh. Duffy) X-linked conditions viz. (G-6 PD deficiency and colour blindness), serum groups viz. (haptoglobulins and transferrins) and other like abnormal haemoglobins and ability to taste phenyl-thi carbamide among 5 endogamous groups of Indian Muslims (Khojas, Bohras, and Misgars and mixed Muslims) was concluded. This study homogeneity of the groups when compared on the basis phenotypic thus indicating inter and intragroup similarities.

on immunoglobulins in disease conditions

In recent years, the starch gel electrophoretic technique of Smithies has helped in resolving many proteins which have been grouped under and alpha-2-globulins. Among these groups, the qualitative resolution of sub-classes of immunoglobulins, i.e. IgG, IgA, IgM and a group of α -2 globulins, known as haptoglobins can be made. Since the introduction of this method in our laboratory, it has been possible to screen the samples for immunoglobulins and haptoglobins.

Immunoglobulins

Serum samples from patients suffering from various clinical disorders, such as recurrent bacterial and viral infections, cirrhosis of liver, collagen disorders and multiple myeloma received from various hospitals, were studied for immunoglobulin patterns. During the year, 344 samples were referred to this department for test. The details of the cases referred and their clinical conditions are presented in the following table :—

TABLE 1

Immunoglobulin pattern in various clinical conditions

	Total	Immunoglobulins			Remarks
		IgG	IgA	IgM	
Bacterial and viral infections ..	260	↓ (65)	↓ (65)	↓ (40)	Remaining cases showed polyclonal hyper-gammaglobulinemia. In one case, monoclonal IgM was found, but there was evidence of increased plasma cells in the bone marrow.
Cirrhosis	40	↑ (32)	↑ (32)	↑ (20)	
Collagen diseases Rheumatoid arthritis.	8	N-(6) ↑	N-(6) ↑	(6) ↑	
Systematic Lupus Erythematosus.	1	N	N	(1) ↑	
Multiple Myeloma ..	35	6 ↑	4 ↑	4 ↑	
Number of cases]	..				

From amongst the cases of multiple myeloma, two cases were followed up for a period of two months since the start of chemotherapeutic treatment. One of the cases, though proved clinically and on bone marrow studies, did not show monoclonal gammopathy. This, on treatment with cytoxan, showed marked improvement in the normal immunoglobulin picture. The other one, which showed clear monoclonal IgG band, also responded to the treatment but no changes occurred in immunoglobulin pattern. No significant changes occurred in paraprotein (M-band) concentration.

Haptoglobins

Serum haptoglobins are the haemoglobin binding α -2 glycoproteins and considered to be of diagnostic value in case of haemolytic anaemia as well as in the necrotic diseases of liver. Originally, the work was started on the genetic types of haptoglobins in various Indian population groups. The starch-gel technique can also indicate the levels of haptoglobin irrespective of genetic type. Thus, when haptoglobin bands fail to be stained with benzidine, the type is termed Hp O-O, which may have significance in relation to the clinical conditions.

Eighty-seven cases suffering from different types of haemolytic anaemia, of the liver (childhood), cardiac prosthesis and malnutrition were from various hospitals for haptoglobin determination. The results the cases studied are given below:—

TABLE 2

Haptoglobins in cases of haemolytic anaemia and other diseases

Clinical condition				Number of cases	Haptoglobin	
					Decreased	Normal
Haemolytic anaemia	..	..	..	35		
Thalassaemia	..	..	..	22	18	4
G-6-PD	..	..	..	2	2	
Others	..	..	..	11	6	5
Cirrhosis (infantile childhood type)	..	..	..	5	5	
Cardiac prosthesis	..	..	..	22	12	10
Malnutrition	..	..	..	25	21	4

Results of thalassaemia and cirrhosis groups showed a large number of with decreased haptoglobin content (less than 20 mg. per cent). study of the results of cardiac prosthesis group showed a high proportion individuals with Hp 0-0 type indicating intravascular haemolysis. cases included both Marasmus and Kwashiorkor.

Studies on specific immunoglobulins

(a) *Anti-tetanus immunoglobulins*.—The project for the immunization of prisoners against tetanus for the production of hyperimmune tetanus antisera has been continued. About 8 litres of plasma have been collected from the hyperimmunised prisoners for fractionation. Further progress was hampered, as the number of prisoners coming forth for the immunisation programme was insufficient. Steps are being taken to increase this number.

(b) *Study of the antitetanus antibody level in the armed forces personnel*.—The armed forces medical authorities were interested in this problem and wanted to study the antitetanus antibody levels amongst this personnel.

As many as 712 samples of sera were received from them and they were screened for the antitetanus antibody level by the haemagglutination. All the samples showed titre much above the minimum titre

necessary for protection from disease. But, none showed more than 2 International Units. Hence, their plasma could not be useful for preparation of human hyperimmune tetanus immunoglobulins, unless they were given booster doses of toxoid.

Anti-D immunoglobulins (IgG).—One batch of plasma of Rh immunised donors has been fractionated to obtain Anti-D immunoglobulins. This is awaiting final testing now.

Studies on Australia antigen

These studies were continued. For screening Au antigen, a new technique like the counter-immuno-electrophoresis (CEP), as recommended by W.H.O., with some modifications, was adopted. Few antigens were referred to the Blood Transfusion Centre, Edinburgh, for confirmation as to their identity. Panel of standard antigens was also obtained from the N.I.H. (U.S.A.) and identity of all our antigens and antibodies was established by testing them against these standard antigens and antibodies. A very strongly reactive persistent antigen (found in the staff member) was referred to Professor Zuckerman of the London School of Tropical Medicine. He also confirmed our findings. Control samples were also obtained from Sydney to confirm our results. The samples from persons suffering from various clinical states were collected and screened for both Au antigen and antibody. The following table gives the findings of these investigations :—

TABLE 3
Incidence of Australia antigen and antibody

Cases	No.	Au antigen + ve Per cent Nos.		Au antibody + ve Per cent	
(1) Normal voluntary blood donors..	200	2	1	..	..
(2) Professional blood donors ..	50	2	4	..	..
(3) Infective hepatitis ..	338	32	11	..	..
(4) Cirrhosis of liver ..	110	3	3	..	..
(5) Lepromatous leprosy ..	90	11	12	..	..
(6) Multiple transfusion cases ..	73	3	4	7	10
(7) Mother and child combination ..	22	..	..	..	..

Purification of Australia antigen was carried out by block electrophoresis and the purified fraction was used for production of antibody to Australia antigen in laboratory animals. Rabbits and Rhesus monkeys were immunised for this purpose. The monkeys showed good response. A paper on this study was presented at the conference of the Asian and Pacific Division of the International Society of Haematology, Melbourne, in May 1971. This paper was accepted for in 'Vox'.

Australia antigen and its relation to liver function test

Serum samples collected from different centres were tested for Australia antigen and antibody. Some of them were estimated for S.G.O.T., S.G.P.T., serum bilirubin and alkaline phosphatase (Liver Function Tests). This work is still in progress.

Biochemical and immunological studies of fibrinogen and cryoprecipitate

Fibrinogen was prepared as fraction I (Cohn) from fresh plasma in small batches. Each batch of fibrinogen was tested for sterility and fibrinogen. Clottable protein was estimated on a weight basis by converting into fibrin which was 60-65 per cent. Purified fibrinogen was by the methods described by Blomback. Preparation of anti-serum in rabbits was started using purified fibrinogen prepared the laboratory.

Thrombin was prepared by fractionating pooled plasma and this solution used in estimating the fibrinogen content of fraction I as mentioned

The cryoprecipitates from fresh human plasma, which are of great value the treatment of haemophilia were processed from the freshly collected

The method described by D. L. Brown was followed, but bottles were used in place of plastic bags, as the former of bottles are not yet available in the country. The precipitates were clinically used in few cases at the K.E.M. Hospital. In these there was very good response (Antihaemophilic globulin level rose from 15 per cent. to 85 per cent. of the normal after administration of these

In order to preserve cryoprecipitates over a longer period, were lyophilised and used clinically. The results showed that lyophilicryoprecipitates were as effective as the frozen ones. Tests for evaluation are being worked out.

on red cell enzymes

Cases of haemolytic anaemia, neonatal jaundice and drug induced haemodisorders were being screened. Screening of glucose-6-phosphate (G-6-PD) was started some years ago and has been continued. other markers, pyruvate kinase (PK) and glutathione of the red cell were added in the past two years to enlarge the battery of for these clinical disorders.

One hundred and sixteen cases of haemolytic anaemia and neonatal were referred for G-6-PD deficiency.

Twelve cases (10 per cent.) were found to be deficient by using the BCB decolorization technique of Motulsky. All affected cases were males.

Six of these deficient cases were assayed quantitatively by using Zinkham's technique. It was observed that all of them had almost 'nil' activity in their red cells. Mothers of deficient children could be clearly categorised as heterozygotes using quantitative assay. Their red cells showed about 1.6 to 1.8 units/ml. RBC activity as against 3.5 units in the controls.

Using the quantitative assay of G-6-PD, our laboratory has tested G-6-PD activity in normal males and females as well as in deficient males and heterozygote females. The findings are given in Table III. It will be observed that the average values of enzyme activity in males, i.e. hemizygotes are comparable to homozygote females. However, the heterozygote females showed much less enzyme activity and could be distinctly classified. Thus, this quantitative assay proved to be very useful in distinguishing homozygote females from heterozygotes for G-6-PD deficiency gain. Since many of the new-born deficient male infants developed neonatal jaundice due to G-6-PD deficiency, our laboratory undertook the studies of quantitative assay in the new-born infants. The findings again suggested that new-born normal males as well as females have as much activity as adults.

PK

Ten cases were referred for the PK deficiency. Clinical features of these cases were hepate and/or splenomegaly anaemia and jaundice, which was persistent in 7 cases. Screening of these samples with the techniques already described (see last year's report) did not show any PK deficiency.

TABLE 4

Normal values of red cell G-P-PD in males, females and the new-borns

Subjects	Number of persons tested	BCB Test	Activity* Quantitative method (above given)	
			Average	Range
Normal males ..	.. 25	Normal (40'—50')	4.2408	2.9516 to 5.530
Normal females ..	.. 15	Normal (45'—60')	3.965	2.400 to 5.30
Male babies ..	.. 9	(40'—50')	4.424	3.095 to 5.982
Female babies ..	.. 9	(45'—60')	3.687	2.765 to
Deficient males ..	.. 8	More than 120'	Zero activity†	
Heterozygote females	.. 6	50'—70'	1.69, 0.737, 1.68 1.84, 1.51, 1.84	

* Activity in units/ml. of packed cells/min.

† Excluding one filaria case which decolorized at 100 minutes.

GSH

The normal values of glutathione levels were studied in healthy males and females. Table IV gives the results of the test. It was observed that the pregnant females have a significant lower level of glutathione. The table also gives the values of glutathione in the new-born male and female infants and the levels in both are comparable to these in adult males.

TABLE 5

Normal GSH levels in males, females and new-borns

Subject		Number of persons tested	Range	Mean value ± S.D.	*
Normal males	..	68	51.04 to 83.60	63.04	± 0.27
Pregnant females	..	25	33.00 to 51.04	41.85	± 6.81
Male babies	..	9	40.48 to 88.44	60.20	± 5.7
Female babies	..	9	54.12 to 98.56	67.6	± 6.2

*In mg. percentage of whole blood.

Glutathione levels were also estimated in six G-6-PD deficient males and the levels ranged between less than 10 mg. per cent. and 32.5 mg. per cent. Three cases had levels of less than 10 mg. per cent., while the levels in other 3 cases were 12.98 mg. per cent., 25.96 mg. per cent. and 32.5 mg. per cent. respectively. These findings suggested that 3 cases with less than 10 mg. per cent. could also be classified as glutathione deficient. Family studies in 2 of these deficient cases showed that mothers in both the cases were G-6-PD heterozygotes and the glutathione level in one case was less than 10 mg. per cent. and other 43.12 mg. per cent. The other case, therefore, appears to be of genetic glutathione deficient type.

Genetical and red cells enzyme studies in Diabetes mellitus

Samples were collected from the diabetic patients in 'EDTA' at the K.E.M. Hospital Diabetic Unit in Bombay.

The following tests were carried out under the title 'Biochemical and Genetical Study of Diabetis' at the Haffkine Institute, Bombay :—

(i) Quantitative estimation of blood sugar and Glucose Tolerance Test by the Somogy's method.

(ii) Blood haemoglobin percentage by 'Sahli HELIGE HEMOMETER'.

(iii) Quantitative estimation of enzyme in the red cells.

(a) *G-6-PD*.—Enzyme estimation is done by the method given by the W.H.O. in the Technical Bulletin Series (1967) 366. (Standardization and procedure for the quantitative estimation of G-6-PD).

(b) *Glutathione*.—Quantitative estimation is done by the method of BEUTIER, E., DURON, O. and Kelly [British Medical Journal of Laboratory and Clinical Medicine (1963) 16 : 882].

(c) *Pyruvate Kinase*.—Quantitative estimation of pyruvate kinase is done by the method of TANAKA *et al* (Journal of Haematology 1963, Vol. 7).

As regards the genetical studies of the red cells, the following groupings have been done :—

A₁, A₂, B, O

Rho

M N

In case of serum, the haptoglobin and transferrin types were checked by gel-electrophoresis.

DEPARTMENT OF IMMUNOLOGY

Stability of purified antitoxic sera

It was reported last year that the residual pepsin in enzyme-digested sera was found to be the cause of deterioration of antibody activity during storage. A slight modification in the process of manufacture of pepsin-digested sera was introduced last year to reduce the pepsin content of processed sera. Samples of such sera were kept in cold storage and at room temperature for stability studies. After 9 months of storage, the samples showed no significant deterioration. Further studies are continuing.

Insolubilisation of pepsin

The use of ethylchloroformate for the preparation of active insoluble pepsin was described last year. Experiments to insolubilise pepsin using gluteral-dehyde were tried. The results were not as encouraging as with ethylchloroformate.

Fractionation of Russell's viper venom and isolation of biologically important constituents

Small quantities of Russell's viper venom were fractionated using starch block electrophoresis. These will be analysed for their enzyme and toxic activities.

in tetanus toxin production

It is well-known that, in the production of tetanus toxin, there is a large from batch to batch of commercial tryptone (tryptic digest of used. Attempts were, therefore, made to study the conditions of casein with trypsin and its further treatment to determine the conditions for maximum toxin production. Preliminary results that the digestion of casein at 50°C at pH 8.2 for 4 hours, followed heating to boiling at pH 4.6 give the best results.

of steroid hormones on antibody production

Sheep R.B.C. was used as an antigen to study the effects of administration of steroid hormones on normal and gonadectomised male and female In male mice, ethinyl oestradiol and methandienone (Dianabol-Ciba) increased antibody production, especially in the secondary In the female mice, in addition to ethinyl oestradiol and methan-progesterone also showed stimulation in the secondary response. ovariectomised female mice, administration of ethinyl oestradiol suppressed the primary and secondary response while the response to methan-was slightly less after ovariectomy. However, in the case of male mice, there was slight enhancing effect of oestradiol, no suppressing effect.

in Indian water buffalo

Six antigenically distinct classes of immunoglobulins were identified in serum using immunoelectrophoretic technique and rabbit anti-immunoglobulin serum. Three of the immunoglobulins had mobility the gamma region and were identified as IgG₁, IgG₂, IgG₃, two immunoglobulins were identified as IgA₁, IgA₂ and the sixth was identified as IgM.

Two antigenically distinct classes of immunoglobulins were observed in concentration in precolostral buffalo calf serum. After suckling, the spectrum of calf serum appeared to be similar to the of colostral immunoglobulins of the dam. These observations that there is direct absorption in undegraded form of colostral by the gut of the neonatal calf. Four distinct immunoglobulins were identified in buffalo colostral whey. These were identified IgG₁, IgA₁, IgA₂ and IgM. The most prominent immunoglobulin in was IgG₁.

Studies were conducted on the transfer of antibodies through the to the new-born calf. Five pregnant buffaloes were immunised three doses of absorbed tetanus toxoid at intervals of about one each, during the last few months of gestation. At the time of the average mean antitetanus antibody titre of serum was

11.8 I.U./ml. The serum of new-born precolostral calf showed no anti-tetanus activity, indicating lack of placental transfer of antibodies. The first colostrum showed an antibody titre of about 31 I.U./ml. (mean value), which was nearly 3 times higher than in the serum of buffaloes. After 24 hours the antibody titre in colostrum was 16.1 I.U./ml. and after 72 hours, it was 2.3 I.U./ml., after one week 0.46 I.U./ml. and after 4 weeks 0.032 I.U./ml. The calf showed highest antibody titre in serum of 2.9 I.U./ml. at 72 hours, after birth.

Studies in diphtheria toxin production in synthetic medium

A synthetic medium, consisting of 5 amino acids (glutamic acid, cystine, valine, isoleucine and methionine), 5 vitamins [nicotinic acid, thiamine, pyridoxine, biotin (or pimelic acid), pantothenic acid (or B-alanine)]; inorganic salts (NaCl , MgCl_2 , K_2HPO_4 and CaCl_2) and maltose, was formulated which could give about 75 to 80 Lf/ml. of diphtheria toxin using *C. diphtheriae* strain PW8-W. Cystine could be replaced by sodium sulphide, thiosulphate or thioglycollate, but not by sodium sulphite or methionine. Methionine could not be replaced by cystine.

The synthetic medium was used for studying the inhibition of toxin production by iron. Iron (0.1 mg.) added as FeCl_3 brought down the toxin produced to less than 5 Lf/ml. The effect of this quantity of iron could be partially nullified by adding excess of calcium and phosphate or by adding chelating agents, such as EDTA and citric acid. It was interesting to note that the vitamin biotin, in a small concentration of 0.003 mg. per 100 ml. was also effective, while the other vitamins were not.

Role of phage in induction of toxin

Phages are known to convert non-toxinogenic diphtheria strains into toxinogenic strains. Thirteen phages received from Roumania and one phage (P) obtained from America were used to study induction of toxin by phage. These 14 phages could be classified into 2 groups depending on host specificity. Phages P, 1, 2, 3, 4, 5, could infect diphtheria strains 1180 and C_7 respectively and phages 10, 11, 12, 14, 15, 16, 18, 24 could infect diphtheria strains 304 and 3148. A strain of diphtheria *C. ulcerans* 603. was also received, which could be used as indicator strain to detect the phage present in PW8 strains. This *Ulcerans* strain could be infected with phages P, 1, 2, 3, 4, 5, 12, 15 and 24.

Another interesting observation was that phage 3 could infect PW8 strain, which already has a phage and such superinfection did not reduce its ability to produce toxin with this strain.

It was also possible to obtain recombinant phages from phages 14-15 and 18-24, which showed different host range from their parent strains. Phage 15, which does not carry toxin marker, was converted into a toxigenic phage after passing through toxin-producing host strain 3148.

Search for drugs effective in experimental tetanus

A number of drugs, such as barbiturates, chlorpromazine, paraldehyde, etc., are used as adjuncts to serotherapy of tetanus. It was not known whether any of these drugs show protective action against tetanus. A number of drugs were, therefore, screened in mice. Among these drugs, vitamin C and chlorpromazine showed very slight protective effect. A combination of equal amounts of neostigmine and atropine was found to be better than all other drugs tested so far. A quantity of 0.025 microgram of each of the two drugs, injected at the same time as the toxin, was effective against about $1\frac{1}{2}$ lethal doses of tetanus toxin. These drugs could save mice from a lethal dose of the toxin when they were administered with a single dose, upto 4 hours later. It was interesting to note that the use of higher amounts of these two drugs was less effective. The reason for this is not known. Similar results were obtained in rats also.

DEPARTMENT OF PHARMACOLOGY

Studies in antileprosy drugs

The Indian Council of Medical Research has sanctioned a Scheme under Dr. M. B. Bhide, M.D., for studies in antileprosy drugs. Ten biopsies of subcutaneous nodules from untreated cases of lepromatous leprosy were collected from all over Maharashtra State. Shepard's method of counting, dilution and inoculation of the lepra bacilli was standardised in the department. The bacilli were injected into the foot-pads of CBA mice—a strain sensitive to leprosy—received through the courtesy of Dr. Karat of Bangalore. The animals were housed in an air-conditioned room, as it is known that good growth of the bacilli occurs at lower temperature. At the end of 6 months, the foot-pads are removed and fixed in formalin. The serial sections will be taken up to observe the growth of the bacilli. In addition to this, thymectomised mice were also injected with the bacilli. In addition, the animals were given ALS intramuscularly at regular intervals.

Screening of drugs for their antileprotic activity *in vitro*

Screening of the compounds for their antileprotic activity *in vitro* by the method, using I.C.R.C. bacillus in Dubos medium was continued. About 100 compounds received from the Central Drug Research Institute were screened and two compounds, bearing code numbers Res. 61/71 and Res. 82/71 were found to be active at a concentration of 400 mcg/ml.

Experimental amoebiasis

The Department of Chemotherapy synthesizes organic compounds with a view to see their anti-amoebic activity. The screening was carried out in this section, using both *in vitro* and *in vivo* methods. The culture of *E. histolytica* was used for infecting rat intracaecally and hamsters intraphepatically. Compound bearing No. R 2289 has shown some promising activity in the preliminary work.

Studies in testing drugs for possible use in the treatment of filariasis

At present, only diethyl-carbamazine is the available drug for the treatment of filariasis. There is need for a more effective drug. With this end in view, the work of testing drugs against filariasis was undertaken. At present, there is no animal model available for testing drugs against human microfilaria. So, the preliminary work of establishing a model was initiated. A colony of the vector *Culex* mosquito was built up. Attempts were made to feed them in the laboratory by using human blood and chicken membrane.

Studies on experimental Giardiasis

In the present work, an attempt was made to cultivate *IN VITRO* *Giardia lamblia*, the causative organism of human giardiasis. The medium used was the one described by Karpetyan (1960), who cultivated *Giardia* for the first time. This medium contained 10 per cent. Hottinger's tryptic meat digest, 5 per cent. chick embryo extract, 25 per cent. inactivated horse serum and 60 per cent. Hank's solution. The medium was modified by the addition of 10 per cent. 'Lab lemco' instead of Hottinger's digest and chick embryo extract. Stool samples containing cysts and trophozoites of *Giardia lamblia* were taken in normal saline. Nearly 2 ml of the sample was inoculated into 10 ml of medium. Also, 1 ml of 1 : 50,000 solution of acriflavine was added. The culture medium was incubated at 37° C. and was observed for the parasites at the end of every 24 hours. The maximum growth was observed at the end of 48 hours of incubation. In this medium, *Giardia* could survive for about one week. Further work on the cultural characteristics of *Giardia* and on animal experiments is in progress.

Screening of drugs against *Ascaridia* (roundworms)

Method of infecting chickens by *Ascaris galli* is being standardized in the laboratory. *A. galli* were collected from intestines of infected chickens. Eggs of *A. galli* were obtained by disruption of the uteri of gravid females. The eggs were cultured to an infective stage in water in petri dishes. After a month, infective larvae were formed. Five hundred eggs were fed to four-week old chickens. The experimental birds are under observation.

Antidiabetic activity of indigenous drugs and synthetic compounds

A series of thiadiazole and oxadiazole compounds, synthesised in the Chemotherapy Department, were screened for their antidiabetic activity in this department. During this screening, three compounds were found to be quite effective as hypoglycaemics in normal rats and rabbits. Compound I (a thiadiazole) and compound II (an oxadiazole) showed promising results at levels of 31.6 per cent. and 30.8 per cent. respectively, at the end of 7 hours at a dose level of 25 mg/kg. But, in glucose pre-treated rats (800 mgs/kg of glucose injected subcutaneously), these compounds failed to show any good activity. Both the compounds failed to produce the same effect (i.e. 31.6 and 30.8 mgs per cent. blood sugar reduction) when the drug was given in a dose of 25 mg/kg daily for 10 days.

Pharmacological and toxicological evaluation of new drugs

The drug No. R. 585 has not shown any toxicity in rats when fed for one month even with a high dose of 1 mg/kg. The following studies were made on the rats: (i) complete blood picture, (ii) liver function tests and (iii) kidney function test. At the end of one month, the animals were sacrificed and all the viscera examined. About 20 tissues from each animal were referred to the Department of Clinical Pathology for histological examination. In all, 40 rats were used in this study. Similar trials in *Macacca radiata* were undertaken and results are awaited.

Assay of Pantothenic Acid

The present method of assay of pantothenic acid is considered defective due to the presence of fatty acids and metallic ions in the formulations.

it was necessary to evolve a new method for the assay of pantothenic and its salts,

The yeast culture, "*Schizo-Saccharomyces pombe*" was selected for use in the assay of Pantothenic acid and its salts. This organism had been used for the microbiological assay of inositol and pyridoxyne hydrochloride. The basal media recommended for these vitamins were taken up for experimentation with different concentrations of the various ingredients of the basal media. The results of growth on this organism were studied and suitable modifications made in the basal media to permit its use for the assay of pantothenic acid in pharmaceutical preparations.

Preliminary studies were carried out on the various ingredients of a large number of pharmaceutical preparations available in the market. The effect of these constituents on the growth of the test organism was studied. In addition, the effect of different pH values, different periods of incubation, different periods and pressure of sterilisation, different quantities of inoculum and other factors involved in a microbiological assay were studied and suitable modifications in the method made. This modified method is now being employed in the microbiological assay of pantothenic acid and its Na and Ca salts and the results obtained are being compared side by side with the standard recommended method, employing *L. arabinosus* ATCC No. 8014.

Studies on haemolytic drugs

Many drugs are known to produce haemolytic reaction in man. The mode of action is not fully understood. Hence the present work was undertaken.

Washed red blood cells were suspended in different concentrations of various drugs, viz. acetanilide, sulphanilamide, nitrofurantoin and quinine sulphate. The concentrations of the drugs were not altered after incubation with cells. Glucose-6-phosphate dehydrogenase deficient cells were suspended in solutions of various drugs. The concentrations of the drugs were measured spectrophotometrically. No absorption of the drugs in the red blood cells was observed.

DEPARTMENT OF VIRAL VACCINES

Poliomyelitis Surveillance

Enterovirus Unit, during the period January to December 1971, recorded 810 cases of paralytic Poliomyelitis in Greater Bombay. The seasonal distribution of Paralytic Poliomyelitis during 1971 showed a rise as compared to the previous years. The period of higher incidence of poliomyelitis being between July and October, during which 57 per cent. (465) cases of the cases were recorded.

TABLE 1

Monthwise distribution of paralytic poliomyelitis cases in Bombay (1971)

Month					Total number of cases	Percentage	Cumulative percentage
January	..	..	..	..	26	3.2	3.2
February	..	..	..	..	23	2.8	6.0
March	..	..	..	..	25	3.0	9.0
April	..	..	..	..	28	3.4	12.4
May	..	..	..	..	44	5.5	17.9
June	..	..	..	..	47	5.8	23.7
July	..	..	..	..	106	13.0	36.7
August	..	..	..	..	135	16.6	53.3
September	..	..	..	..	114	14.0	67.3
October	..	..	..	..	110	13.5	80.8
November	..	..	..	..	78	9.6	90.4
December	..	..	..	..	74	9.1	99.5
Total					810		

The disease continues to be an infantile paralysis affecting mainly the children between the age group of 6 months and 2 years (Table 2). During 1971, as many as 70 per cent (573 cases) of cases were recorded in children below 2 years. Children below 5 years accounted for 96.6 per cent. of the total cases. Five cases were recorded between the age group of 14 years and 20 years. The 60 days' follow-up of these cases showed that 2 cases (both 20 years) had residual paralysis after 60 days, while the remaining cases recovered completely. The details of the investigation of the status of residual paralysis after 60 days are given below :—

Poliomyelitis Surveillance

Status of residual paralysis after 60 days (1971)

1. Paralytic poliomyelitis cases with residual paralysis after 60 days—
 - (a) severe residual paralysis ... 67
 - (b) partial residual paralysis ... 360
2. Paralytic poliomyelitis cases without report on residual paralysis. 203
3. Fatal cases ... 53
4. Paralytic Poliomyelitis cases without residual paralysis (complete recovery). 127

Paralytic illness amongst vaccinated children

During the year 1971, 112 paralytic polio cases amongst vaccinees were recorded. Complete virological studies could be done in 107 cases. Inadequate immunization was detected in 59 cases, while 53 individuals had received all the three doses of live oral polio vaccine. Age distribution and seasonal distribution of these cases does not show any significant difference when compared with total number of cases recorded in the year 1971. The virological results indicated infection with all the three types of polioviruses. It showed the presence of virus in 73 cases out of the 107 cases taken up for study. Further studies are in progress.

TABLE 2
Agewise distribution of paralytic poliomyelitis, Bombay (1971)

Age group in years		Total Number of cases	Percentage	Cumulative percentage
0-6/12	..	58	7.1	7.1
6/12-1	..	249	30.7	37.8
1-2	..	324	40.0	77.8
2-3	..	113	13.9	91.7
3-4	..	27	3.3	95.0
4-5	..	12	1.4	96.4
5-6	..	8	0.9	97.3
6-14	..	14	1.7	99.0
14-20	..	5	0.6	99.6
Total		810		

Poliomyelitis in Delhi

During the summer of 1971, the capital was in the grip of an epidemic of paralytic poliomyelitis. The Government of India and the Indian Council of Medical Research requested help of the Enterovirus Unit of this Institute for investigations. Dr. K. H. Dave, Assistant Director, Department of Viral Vaccines, was deputed to carry out the necessary investigations. Samples from 93 cases of paralytic poliomyelitis were collected for virological and serological studies.

Laboratory investigations carried out on these materials indicated that the epidemic was caused by poliovirus type 1. The epidemiological features of this epidemic were found to be similar to those observed in Bombay.

Haffkine Institute supplied 1,46,625 doses of trivalent oral polio vaccine to the Government of India to combat the

Enterovirus studies in rural population (Sirur Taluka)

The Enterovirus Unit in the past has concentrated its activity in urban areas. It is now considered essential that the data obtained in urban areas be compared with similar studies in rural areas. Beginning from February 1970, the Enterovirus Unit, in collaboration with the Directorate of Public Health, State of Maharashtra and the Department of Social and Preventive Medicine, B. J. Medical College, Poona, has drawn up a programme for enterovirus studies in the rural areas. This study has been started with the following objectives :—

- (1) to study incidence and spectrum of enterovirus infection in the rural child population,
- (2) to study the extent of natural immunisation against poliovirus in the rural population, and
- (3) to study the efficacy of oral polio vaccine in the rural population.

For the above studies, 4 villages in the Sirur Taluka (Poona District) have been selected. All the four villages consist of stationary agricultural

. Work was started in February 1970. A team of 5 workers the Enterovirus Unit was stationed at the Primary Health Centre,

Data and materials were collected by house-to-house visits. of materials for enterovirus survey and survey of antibody against poliovirus have been completed.

For virological survey, sterile containers were distributed by door-to-door Mothers were explained the method of collection of stools. The were collected on the following day.

For the collection of blood samples for serological studies, difficulties often encountered in the field in terms of non-cooperation of the and inadequate facilities for collection of blood by venepuncture.

Enterovirus Unit has now standardised a simpler technique in which capillary whole blood could be collected on filter papers which be subsequently used in the laboratory for antibody estimations. sirur, wherever difficulties were experienced in collecting blood by finger-prick technique was used. As a result of this, refusals the participants were comparatively fewer.

For enterovirus survey, materials from 952, out of a total of 1316 children the age of six years, were collected and tested. As many as 290 were found to be excreting an enterovirus. Identification of the showed a wide spectrum. In all the three types of polioviruses 1 types of Echoviruses and 11 types of Cocksackie viruses were encountered the survey. Details are presented in Table 3.

Antibody survey for poliovirus indicated that approximately 50 per cent. the child population did not have antibodies against polioviruses.

TABLE 3

Sirur Virus Studies

Data	Narayangaon	Karda	Ranjangaon	Malthan	Total
1. Total focal samples studied.	147	187	250	337	921
2. Samples positive ..	30	46	79	135	290
3. Viruses identified so far	P 1—0* P 2—0 P 3—3 CA 2—1 CA 5—2 CA 6—1 CA 14—1 CB 3—1 E 2—1 E 9—2 E 14—1	P 1—3 P 2—0 P 3—1 CA 1—1 CA 4—16 CA 9—2 CB 2—2 E 12—1 E 13—2 E 14—1 E 19—1	P 1—2 P 2—2 P 3—2 CA 2—1 CA 4—11 CA 5—1 CA 9—1 E 4—2 E 5—1 E 6—1 E 7—18 E 15—1 E 19—3	P 1—2 P 2—10 P 3—4 CA 1—3 CA 2—5 CA 4—7 CA 5—0 CA 6—7 CA 9—1 CB 1—1 CB 2—3 CB 3—1 CB 4—4 E 1—3 E 7—15 E 9—3 E 11—1 E 13—1 E 14—2 E 19—16	P 1—7 P 2—12 P 3—10 CA 1—4 CA 2—7 CA 4—34 CA 5—3 CA 6—8 CA 9—4 CA 14—1 CB 1—1 CB 2—5 CB 3—2 CB 4—4 E 1—3 E 2—1 E 4—2 E 5—1 E 6—1 E 7—33 E 9—5 E 11—1 E 12—1 E 13—3 E 14—4 E 15—1 E 19—20

* P 1—Poliovirus type 1, CA 1—Coxsackie virus A Type 1,
CB 2—Coxsackie virus B Type 2, E 1—ECHO virus type 1

Haemagglutination studies

All the virus isolates obtained in tissue culture were tested for haemagglutination activity using human 'O' erythrocytes. A total of 195 isolates were tested in which 46 isolates were found to possess HA activity. Using haemagglutination inhibition test, it was possible to identify 30 isolates (Echo 3-1, Echo 6-2, Echo 7-17 and Echo 19-10).

Marker studies on Poliovirus isolates

During the enterovirus survey in the child population of Sirur Taluka, a total of 29 children out of 921 were found to excrete poliovirus. All the three types of polioviruses were encountered with a slight predominance of type 2 poliovirus (Polio 1-7, Polio 2-12 and Polio 3-10). There has been no immunisation programme in Sirur Taluka so far.

All the 29 polioviruses isolated from the children at Sirur have been studied so far by genetic marker test. Reproductive capacity at higher temperature (40°C) test has been performed on these 29 isolates. Out of 7 polioviruses type 1 studied, only 1 showed wild character, 3 showed intermediate character while the remaining 3 showed attenuated character. Of the 12 polioviruses type 2, 6 showed wild character, 4 showed intermediate character, while 2 showed attenuated character. Out of 10 polioviruses type 3 studied, 6 showed wild character, 2 showed intermediate character and 2 showed attenuated character.

Paralytic poliomyelitis in Sirur

While surveying the four villages for enterovirus study, attempt was made to find out the incidence of paralytic poliomyelitis in the local population. During the last 6 years (1965 to 1970), 9 cases of paralytic polio had occurred in the local child population.

The incidence of paralytic poliomyelitis in these four villages selected for the present study, works out to : 1 case per 7000 population per annum. The incidence of paralytic poliomyelitis in an urban area like Bombay is approximately 1 case per 10,000 population per year.

Acute Conjunctivitis in Bombay

In the year 1971, there was an outbreak of kerato conjunctivitis in Bombay. Entero-virus Unit collected eye swabs from 153 cases. The Unit could not isolate any viruses either in monkey kidney tissue culture, Hela culture or in suckling mice (16 to 24 hours old).

Study of simian intestinal viruses

It is known that a large group of viruses inhabit different tissues of the

It was found that as many as 62 monkeys out of 100 *M. mulatta* (Rhesus) monkeys harboured cytopathogenic agents in the intestinal tract. These isolates showed presence of two types of agents, one group showing adenovirus type of cytopathic effect (27 strains) and the other showing human enterovirus type of cytopathic (CPE) effect (33 strains).

Identification of these viruses is in progress. So far, two serotypes, SV17 and SV33 have been detected amongst adeno type of cytopathic agents.

In addition to the above work, 50 faecal samples were collected from *M. radiata* (Bonnet) monkeys to study the presence of viral agents. It was possible to isolate 26 agents from these 50 samples. Of these, 14 manifested C.P.E. like adeno type, while the remaining (12) showed CPE like human enterovirus type.

Antiscrum has been prepared against one locally isolated adenolike strain. Further work is in progress.

Study of response to oral polio vaccine (OPV) in children

In view of the apparently increasing incidence of paralytic poliomyelitis amongst the vaccinated children, this study has been undertaken to find out the efficacy of oral polio vaccine.

Children between the ages of 3 months to 2 years have been selected for this study. Pre-vaccinated blood samples, stool samples and rectal swabs are being collected.

Pre-titrated vaccine is administered in 3 doses at an interval of 6 weeks between each dose. Stool samples are collected one week after the administration of each dose. Two weeks after the final dose, the blood is collected for post-vaccination test. The administered vaccine is re-titrated to find out its efficacy.

So far, 63 babies have been enlisted for this study. Thirty children have completed the immunisation schedule. It has been possible to collect the necessary samples for virological and serological studies from 22 children. Others are yet to complete the immunisation schedule. Fresh enrolment of children for this study is in progress.

Forty-one samples of oral polio vaccine, used during this study, have been re-titrated after the vaccine was administered to the children. All the samples were found to be of adequate potency.

DEPARTMENT OF VIROLOGY

Pathogenesis of Rabies

The results obtained in brown fat studies, using K.F.D. virus as a parallel infecting agent, were confirmed. At the same time, an attempt was made to study the presence of virus in the local muscle tissue. Mice were inoculated in the left thigh, as described in earlier reports and two mice from each group were sacrificed daily after 48 hours infection. In addition to the brain, brown fat and local muscle tissue at the site of injection, muscle tissue from the contralateral site were also taken, processed in a similar manner and a 10 per cent. suspension inoculated intracerebrally into six mice for each tissue harvested. Unfortunately, the infecting dose of CVS virus was rather low and very few of the original mice appeared to be infected with rabies. Only one CVS mouse, harvested on the 7th after infection, showed signs of paralysis and tissues from it were positive for virus isolation. On all other days, the mice appeared normal and no virus was isolated from the brain or any other tissue. From the mice (one paralysed and the other normal) sacrificed on the 7th day, however, all pooled tissues, including the contralateral muscle, were positive for the virus. At this stage, the infection of the CNS and onset of symptoms had already occurred.

Hence, the presence of virus in the contralateral muscle can be interpreted as due to retrograde diffusion from the CNS. The persistence of rabies at the site of inoculation, prior to CNS involvement, cannot, however, be ruled out, since the infecting dose in this experiment was too small and the quantity of residual virus, if any, might have been too small for detection. This work is being continued.

tests for rabies

The importance of quick and reliable diagnostic tests is evident. Anti-treatment is prolonged, laborious and costly. It also involves some and risks to the patient. The necessity of treatment depends on presence of rabies in the biting animal, normally the dog. Hence, is the utmost importance that methods of diagnosis should be improved.

Complement fixation test.—Laboratory diagnosis today consists of direct staining or (b) fluorescent staining and microscopy of smears and animal inoculation. In conditions prevailing in our country, the first tests are frequently unsatisfactory, as the brains may be received in condition, etc. Reliance has, therefore, to be mainly placed the mouse inoculation test, which normally takes about 15-21 days. The workers have reported a complement fixation test, which they claim be as reliable as the mouse inoculation test and can give the results within hours. Further, it can be elicited even on decomposed specimens. It

was, therefore, decided to try the complement fixation test as a method of rabies diagnosis and compare it with the other procedures in use for speed and reliability.

Standard antigen was prepared from fixed virus sheep brain suspension and standard antiserum was prepared by immunization of guinea-pigs as described by Ando *et al.* Tests with standard antiserum and antigen prepared from 3 different fixed virus sheep brain suspensions were put up. The titre of the standard antigen was 1:2 to 1:4. This is considered as too low for standardization of the test. Fresh standard antigen is being prepared for study.

The Haemagglutination test was attempted, using Paris strain and HEP flury virus as antigen and cells of one day old chicks.

The flury virus was obtained as a lyophilized 33% suspension from the Indian Veterinary Research Institute, Izatnagar. Paris strain virus was obtained from sheep brain infected with the virus, having a mouse infectivity titre of $10^{6.7}$ LD₅₀. Virus was diluted in 0.4 per cent. bovine albumin borate saline at pH 9.0. The chick erythrocytes were obtained from one day old chicks from the Haffkine Institute poultry farm at Pimpri and were suspended in phosphate-buffered saline at pH 6.4. The test was carried out in micro plates (U type), using 0.025 ml. of antigen dilution, 0.025 ml. of 0.4 per cent. BABS and 0.05 ml. of cell suspension. The plate was incubated at 0° C. on ice slurry for 1 hour. No agglutination was obtained with the virus suspensions and chick cells used.

Fluorescent antibody.—Smears from brain and salivary glands of 100 normal dogs, studied for carrier state described in the previous report, as well as impression smears from all specimens sent for rabies diagnosis, have been fixed in cold acetone and preserved in the cold. The Zeiss condenser ordered for this purpose, which was held up by the Customs authorities, has now been released. The work is continuing.

Epidemiology of Rabies

Rabies from human patients.—One patient was added to the series during the year. The saliva, tongue scraping and urine of this patient were examined for rabies. No virus was isolated. Blood sample was not obtained.

Blood samples of patients collected last year were tested for neutralizing antibodies against rabies. Two of these had a history of dog bite more than one month ago and had taken no antirabic treatment. One patient

had dog bite 21 days earlier and had taken 14 injections of Antirabic vaccine starting 2 days late. All the three patients died within 2 days of admission to the hospital.

No demonstrable antibody (titre of 1 : 5 or above) was found in the sera of the former two patients, while serum of the third one, who had received a course of vaccine, showed neutralizing antibodies to rabies in a titre of 1 : 80.

Influenza surveillance

Isolation.—One throat washing was collected from a suspected case of influenza. No virus was isolated from the throat washing.

Serology.—Sera of persons from different age groups, collected at various times by the department, or from blood donors and volunteers, were obtained for study of influenza antibodies in an effort to gain some insight into the history of the successive influenza infections in the city. The sera obtained were as follows :—

Pre-1968	145
1969	156
1971 (early)	283
1971 (August)	43
1971 (September)	31

In addition, 42 samples of mother and cord blood (pairs), collected in 1967, were also available for study.

The age groups represented varied from 0-6 months, upto 91-100 years. The HAI test, using a micro technique, is being performed and all sera are being tested against 9 representative strains from various endemic and epidemic outbreaks of the last 40 years and against 2 equine strains of influenza. Four hundred sera have been tested so far. The results will be analyzed when the tests are completed.

Elimination of non-specific inhibitors in sera for HAI test.

The A₂/HK/1/68 strain of influenza showed a different pattern of sensitivity to non-specific inhibitors when compared with the previous strains of Influenza. The KIO₄ method of treatment, used for sera, was not found very effective in removing all the non-specific inhibitors. Hence, several comparative tests of different methods were done for the A, A₁ and A₂ prototypes on a few normal animal and human sera.

The results on A₂/HK/1/68 are given below:—

TABLE 1
Normal Sera (reciprocal HAI titres)

Methods of treatment			Horse	Rabbit	Guinea-pig	Fowl	Human
Heat Inactivation at 56°C for 30 minutes.			1,280	..	..	20	40
KIO ₄ + Trypsin	..	..	40	>320	>320	40	80
KIO ₄	..	..	40	>320	>320	40	40
Kaolin	..	..	40	<10	<10	<10	<10
RDE (purified)	..	..	5120	<10	320	<10	<10
KIO ₄ + RDE	..		10 or <10	..	..	..	..

It is clear from the above table that kaolin is the best method of treatment for almost all sera except that of the horse in whose case, the only effective treatment i.e. RDE followed by KIO₄. RDE treatment was found to be the most ineffective treatment for horse and guinea-pig serum although a crude agar filtrate was more effective than purified RDE.

Further, both kaolin and RDE were used simultaneously in several tests for immunised fowl and human sera. It was found that kaolin acted sometimes in a haphazard fashion. As the removal is achieved by physical adsorption, some immunoglobulin fractions (anti-body fractions) were also removed yielding inconsistent results. Moreover, kaolin and distilled water form only a suspension, giving rise to dilution errors in the finally treated serum, inspite of proper mixing.

The work on serology mostly centres round fowl and human sera and, hence, RDE was selected as the method of treatment. As reported by Hoyle (Influenza monograph 1969) the crude filtrate has the ability to remove both alpha and beta type inhibitors and gamma inhibitors in low titres. Hence, this RDE could be conveniently used for any influenza strain with the minimum errors.

Preparation of RDE filtrate

A strain of 4Z Ogawa vibrio (obtained from W.I.C. London) was used. Preparation was initially tried in a liquid medium (Brain heart infusion broth by the House method as recommended by the W.I.C.), but this was not found to be very satisfactory. After trying various alternatives, the best results were obtained with a preparation of RDE by the method of Burnet and Stone (1947) on agar kept at pH 6.9. The filtrate thus obtained was titrated by the method of House (as recommended by W.I.C.). Suitable dilutions were prepared and each dilution was tested with human and fowl sera of known normals as well as immunized before use in the HAI test.

Seroepidemiology of measles

In previous reports, evidence was presented to show that the history of measles, as given by the parents, is generally reliable. The importance of this finding is that it enables us to obtain data on measles by using questionnaire type surveys. Hitherto, such a procedure was not favoured, as it was felt that, unless supported by evidence of antibodies in the blood, such information would not be reliable.

To investigate this reliability further, a study was carried out on sera collected in Pune and adjacent communities. Sera, collected in Pune by the Department of Viral Vaccines for enterovirus studies, were made available for this study. The history of measles was recorded in each case. Seventy-five of these sera have been tested so far. The study is continuing.

Subacute Sclerosing Panencephalitis (SSPE)

In previous years blood samples from cases of this condition were referred to us for estimation of antibodies to measles, since the measles virus was a suspected aetiological agent of this condition and antibody titres against measles of a very high order were found in some of these cases. However, it is now felt that this disease has a more complex aetiology and does not occur solely due to infection of the Central Nervous System by the measles virus. Further, typical cases of this condition are seen, which do not show any evidence of measles aetiology. Various immunological mechanisms of production of this syndrome have been postulated and tests to exclude some of these possibilities are being investigated in co-operation with the clinicians concerned. The cases were screened by the clinicians concerned and only those cases showing a progressive degenerative pattern, with typical EEG and colloidal gold curve, were included in the study.

A total of 33 sera and 7 CSF samples from 30 cases of SSPE were received, of which 18 blood and 7 CSF samples were tested during the current year. Details of these tests are given in the table below:

TABLE 2

Measles antibody titres in sera from SSPE Patients

Case No.	Age	Sex	History of measles (Age)	Interval between onset of measles and onset of neurological symptoms	Duration of neurological symptoms	Antibody titre*		
						HAI	CF	N
33	9 years	M	7 years	2 years	2 months	160	64	256
38@	7 years	M	6 years	1 year	3 months	160	4096	256
38†	7½ years	M	6 years	1½ years	9 months	40	1024	64
39	5 years	M	No		1 month	320	128	512
40	10 years	M	No		2 months	640	4096	1024
41	10 years	F	No		13 days	320	128	512
43	6 years	M	No		2 months	80	1024	128
44	8 years	M	No		8 days	320	8	512
44†	8½ years	M	No		6 months	320	2048	512
45	9 years	M	3 years	6 years	15 days	160	4096	256
47	13 years	F	2 years	10 years	3½ months	80	1024	128
48	2 years	F	1½ years	6 months	6 months	320	1024	512
49	9 years	F	1½ years	7½ years	4 months	80	<4	128
49†	9 years	F	1½ years	7½ years	5½ months	5120	2048	4096
50	9 years	F	No		6 months	40	1024	64
53	10 years	F	No		4 months	160	1024	256
56	8 years	F	No		3 months	1280	4096	2048
57	11 years	M	No		4 months	2560	4096	4096

* Antibody titres are reciprocals of serum dilutions.

@ Second follow-up serum samples.

† Third serum samples.

TABLE 3

Measles antibody titres in C.S.F. from SSPE patients

Case No.	Age	Sex	History of measles (Age)	Interval between onset of measles and onset of neurological symptoms	Duration of neurological symptoms	Antibody Titre*		
						HAI	CF	N
32a	4½ years	F	4½ years	3 months	15 days	20	4	32
36a	8 years	M	No		1 month	40	16	64
37a	20 years	M	2 years	18 years	2 months	320	128	256
39a	5 years	M	No		1 month	<5	<8	4
47a	13 years	F	3 years	10 years	3½ months	<5	<16	4
50a	9 years	F	No		6 months	<5	<8	4
53a	10 years	F	No		4 months	<5	<32	4

*Antibody titres are reciprocals of C.S.F. dilutions.

The following conclusions may be drawn from the data presented:—

1. All cases (satisfying the above criteria of SSPE) do not show high levels of serum antibodies against measles.
2. The presence of measles antibodies in the CSF, which is considered to be strongly in favour of an infection of the CNS, occurs only in some cases (but not in all) in whom the serum antibodies are significantly raised.
3. Elevation of CF antibodies in the serum is more evident in SSPE than in HAI or N antibodies, suggesting a very recent antigenic stimulus.
4. Some cases, which do not show a significantly raised antibody titre at the outset, may develop a high titre after some time, as seen from the results of the examination of repeat samples.

These facts suggest that elevation of circulating measles antibodies is relatively late manifestation in this condition.

Small-pox vaccination

The optimum time for primary vaccination is considered to be between 3 and 6 months of age, to avoid any inhibitory effect of circulating maternal antibody. However, since parents rarely report for vaccination of the child, many of these children, in rural areas, remain unvaccinated. To avoid this, some centres follow the practice of vaccinating new-born children before the mother is discharged from the hospital. The response to vaccination, as judged by the "take", appears to be satisfactory, but it has been argued that, in these infants, the immunity developed may be both inadequate and short-lived.

To resolve this question, a study was planned at the instance of the Department of Pediatrics, Seth G. S. Medical College, Bombay, wherein blood samples were collected at different time intervals from children vaccinated at birth and from children vaccinated between 3 and 6 months of age, at different time intervals, for comparison of antibody titres in the two groups. It is now proposed to collect blood samples from the mother at delivery and from the child at birth as well as before 1 month, 6 months, 1 year and 2 years after vaccination. All the samples will be tested for antibodies to vaccinia by the HAI test.

A further interest in this study is to compare the incidence of vaccinia in the two groups. The incidence of this complication may lower after neonatal vaccination if such complications have, in fact, increased after introduction of the current high-titre vaccine.

The following blood samples have been collected so far :—

Mother's blood at delivery	...	...	335
Cord blood (pre-vaccination)	...	...	335
One month after vaccination	...	...	120
Six months after vaccination	...	...	20

Efforts are under way to improve the follow-up of cases for study. of capillary blood collected on filter discs is being standardized. This make the collection of repeat samples easier.

Rats as vectors of human viral disease

Rats trapped in rural and in urban houses were studied for evidence of human pathogenic viruses as described in previous reports. During the past year, 69 further specimens were studied. Results of isolation studies were as follows:—

Specimens negative (yielding no viral agent) in infant and adult mice or MKTC.	...	...	44
Specimens negative in infant and adult mice under study in MKTC.	...	...	25

Two viral agents, isolated earlier, were studied for haemagglutinating activity with red cells in the following species:—

Guinea-pig, rabbit, hamster, mouse, rat, fowl and sheep.

The test was carried out using mouse brain suspensions at different pH values ranging from 6.2 to 8.00. Both the viral agents showed agglutination activity in sheep and guinea-pig cells. The best titres were obtained at pH 8.00 after incubation at 37° C. for 4 hours, against sheep and guinea-pig RBC, viz. 1:16 and 1:4 respectively.

“ Sixty-one blood samples from persons living in the area under study (Palaspe Village) were collected to test their antibodies to the EMC virus strains isolated. The study is continuing.

Rubella

Twenty-eight suspected cases of Rubella infection or congenital Rubella syndrome were referred to this department for investigation. Twenty-eight blood samples and 18 throat swabs from these cases and 18 blood samples and 11 throat swabs from contacts were collected. Virus isolation from acute samples was tried in cultures of Primary Human Amnion. No viral agent was isolated.

Blood samples tested in HAI included 76 (37 positive, 33 negative and 6 doubtful) collected in the previous year in addition to 86 (47 positive, 37 negative and 2 doubtful) repeated from earlier ones.

Interference between rabies fixed virus (RV) and Rous sarcoma virus (RSV)

Studies on the mechanism(s) underlying interaction between rabies virus, non-oncogenic rhabdovirus and Bryan Master standard strain of RSV, an sub-group A leukovirus in chicks, were continued.

During the course of investigation it was found that blockade could not demonstrated in fresh chicks from the Pimpri Farm. It was subsequently that these chicks were from the new breeding stock (Hy-Line Park)

purchased recently from a commercial supplier and hence, an alteration of the avian leukosis complex spectrum might have occurred in this flock. Subsequent experiments were performed to reproduce the results in the Australian strain of white leghorn chicks supplied by the Government Regional Poultry Farm, Aaray Colony. Time effect curves of the RSV blockade in this flock were almost identical with those seen in the original Hy-Line Park strain, i.e., the effect was short-lived (4-6 days) and biphasic. The reduction in the effect ("dip"), which had been noted at +24 hours in Hy-Line Park strain, was seen at +48 hours in the Australian strain of chicks. This was repeatedly demonstrated.

As reported earlier, the system was suspected to be mediated by interferon on the basis of effect of hydrocortisone and actinomycin D, wherein complete reversal of blockade was seen.

Sera were collected from 5 to 7 days old birds at 24, 48 and 72 hours' intervals following RV inoculation (RV sera). The absence of virus in the sera was demonstrated by intracerebral inoculation of the sera in adult mice. No mouse succumbed to rabies. Control sera (NRB sera) were prepared by bleeding chicks similarly inoculated with normal rabbit brain (NRB) suspensions. Mixtures of each of these sera and RSV were inoculated into the wing web of chicks. It was found that 42 per cent., 13 per cent. and 42 per cent. of the chicks remained tumour-free after inoculation with mixtures containing RV sera of 24, 48 and 72 hours respectively. No blockade was seen with mixtures containing NRB sera. The close correlation found between the interviral blockade and the serum inhibitory activity make it apparent that the blockade is mediated by a serum factor induced by RV.

Interferon is usually active against a wide range of viruses, is species specific, pH stable (pH 2.0 for 1 hour), heat stable (60°—70°C for 1 hour) and shows very low activity in crude preparation. Preliminary experiments with crude serum preparations showed that the substance was heat (56°C for 1 hour) and pH (pH 2.0 for 1 hour) labile. Furthermore, the maximum inhibitory activity corresponded with the peak fraction off a DEAE cellulose column and monitored at 280 m μ . The dialysed fraction was again temperature and pH labile. When mixed with RSV and inoculated into Hy-Line Park chicks, the dialysed fraction failed to show blockade. These data suggest that the serum factor is probably protein in nature but is not an interferon.

Experiments are now in progress to characterise this serum factor and to observe its effect on RNA dependent DNA polymerase—"reverse transcriptase" enzyme of the RSV stock virus used.

TABLE 4

Correlation between inter-viral blockade and serum inhibitory activities at 24, 48 and 72 hours in Australian strain of white leghorn chicks

Experiment	Injection		Time delay	Number of chicks	Percent tumour-free chicks on day—21
	1st	2nd			
1	.. RV	.. RSV	.. 24	12	24
	NRB	.. RSV	.. 24	11	0
2	.. RV ₁	.. RSV	.. 48	15	7
	NRB	.. RSV	.. 48	10	0
3	.. RV	.. RSV	.. 72	16	36
	NRB	.. RSV	.. 72	10	0
4	.. RV serum (24 hours)	.. RSV	.. 0	12	42
	NRB serum (24 hours)	.. RSV	.. 0	11	0
5	.. RV serum (48 hours)	.. RSV	.. 0	15	13
	NRB serum (48 hours)	.. RSV	.. 0	10	0
6	.. RV serum (72 hours)	.. RSV	.. 0	12	42
	NRB serum (72 hours)	.. RSV	.. 0	10	0

DEPARTMENT OF ZOONOSIS

Plague epidemiological studies

Plague epidemiological studies were carried out in 'A', 'D' and 'E' ward areas of Greater Bombay. *R. rattus*, *R. norvegicus* and *B. bengalensis* together constituted the predominant commensal rodent fauna. Of the three principal species, *B. bengalensis* was found to be predominant.

In all, 2470 rats were examined from 'A', 'D' and 'E' Ward areas. *cheopis* was predominant in 'D' and 'E' ward areas and in 'A' ward, *X. astia* showed its preponderance over *X. cheopis*. The studies in 'D' 'E' ward areas are in progress.

Susceptibility status of oriental rat flea to different Insecticides

Assessment of susceptibility status of oriental rat flea, X. cheopis, from Nasik District to D.D.T.—Assessment of susceptibility status of oriental rat flea to D.D.T. and other insecticides revealed a high degree of resistance among fleas to D.D.T. The fleas also showed some tolerance to malathion and fenthion. It was interesting to note that Fenthion was never used in Nasik area for insecticidal purposes.

Susceptibility of Culex P. fatigans to insecticides

Evaluation of susceptibility status of *Culex P. fatigans* from different localities of the Bombay city to different insecticides is in progress. So far, the findings showed a high degree of resistance to D.D.T. and Dieldrin.

Laboratory evaluation of anti-coagulant rodenticides

Periodic assessment of susceptibility status of commensal rodents, viz. *R. rattus*, *R. norvegicus* and *B. bengalensis*, collected from different wards of the Bombay city had indicated the existence of anti-coagulant resistant strains of *R. rattus*. Therefore, rodents from different wards were examined for assessing their susceptibility to anti-coagulant rodenticides. The studies carried out so far reveal the possibility of presence of tolerance among *B. bengalensis* to anti-coagulant rodenticides in the 'D' ward area.

Epidemiological studies in Giardiasis

Giardiasis, caused by an intestinal protozoan *Giardia intestinalis*, rates second to Amoebiasis in intestinal parasitic infestations. It may give rise to asymptomatic carrier state or to severe malnutrition with diarrhoea, specially in the lower age groups.

Stool and serum samples of persons suffering from Giardiasis were collected from the various city hospitals. Axenic cultivation of these protozoa was done by using various media to assess their longevity *in vitro* and for the preparation of a purified antigen.

Studies in Leptospirosis

In all, 526 sera were studied for leptospiral agglutinins. These sera were collected from horses, buffaloes, stray dogs, aborted cases of sheep and buffaloes, aborted women, suspected jaundice and leptospiral patients and children. Thirty-nine sera were found to contain leptospiral agglutinins. The serotypes found were *L. icterohaemorrhagica*, *L. ballum* and *L. pomona*. The titre was found to range from 1 : 30 to 1 : 1000. The tests employed were the fluorescent antibody technique, the haemagglutination test, microscopic agglutination test and sensitized lysis test.

Epidemiological studies in filariasis

The filaria survey, initiated in the municipal areas of the Thana city in March 1971 was completed. Fourteen-thousand two-hundred and thirty-seven night blood smears were collected and examined for microfilariae. Out of 14237 smears examined, 554 were positive for microfilaria. In 212 persons, clinically established filariasis was noted. The rate of live infection and the rate of filarial disease in that area worked out to 3.11 per cent and 1.44 per cent respectively.

Studies on Immunological or Serological diagnosis of Filariasis

HA test (haemagglutination test) to diagnose filarial infection *in vitro* was standardised.

Skin tests with FST antigen obtained from the W.H.O. were conducted on 1055 persons as shown in the table given below :—

TABLE 1

Group	Category of cases	Total cases tested	Results		Remarks
			+ve	-ve	
I	Disease +ve .. .	39	35	4	+ve in 89.74 per cent
II	Microfilaraemics ..	208	139	69	+ve in 66.82 per cent
III	Contacts ..	469	211	258	+ve in 44.99 per cent
IV	Non-contacts ..	339	141	198	+ve in 41.29 per cent

Studies in animal filariasis

Studies to assess the incidence of filariasis in animals and to evaluate zoonotic importance are in progress. Three-hundred and eighteen dogs examined. Out of these, 29 showed the presence of microfilaria in

Horses, sheep and monkeys were the other animals examined. of these, only horses showed the presence of microfilariae in blood. work was recently started and is in progress.

PART III

ROUTING AND MISCELLANEOUS ACTIVITIES

DEPARTMENT OF ANALYTICAL AND CONTROL

The Analytical and Control Department continued to function mainly in the old Nutrition Department during the period of report. Under the prevailing conditions, it was not possible to bring about unification of all the varied functions of the department at one single premises, but the present set-up is a great improvement upon what it was a couple of years back and it is in the continuous process of gradually evolving into a full-fledged Quality Control Department. Ceaseless attempts are being made for its adequate expansion and perfection.

The Department has dauntlessly carried on its activities of examining and certifying all the finished products manufactured by the Institute for their quality, purity and strength, thus fulfilling the drug control obligations.

The sphere of activities of the department expanded with the additional responsibility of taking over analysis of raw materials that are used for the manufacture of chemotherapeutic and biological products. With the meagre staff of the Raw Materials Section, more than 800 samples involving more than 8,000 exacting tests for assays limits for impurities, etc. were done.

Though this Department is at present equipped and staffed to analyse/test finished products only, oft and on, cooperation and help was rendered to production departments by undertaking testing of intermediary products and this pressure was more during the national emergency.

Dr. F. L. Saldanha took over as Assistant Director Incharge Analytical and Control Department on 19th April 1971 after being selected by the Maharashtra Public Service Commission. Shri K. G. Anantanarayanan, who was officiating as Assistant Director, Incharge Analytical and Control Department, was re-transferred to Pharmacology Department as Senior Scientific Officer, Class I.

Shri N. Ramani, Senior Technical Assistant, who was promoted and transferred to Blood Bank as Junior Scientific Officer was re-transferred as Junior Scientific Officer to this Department. Kum. U. D. Bapat joined the department as Senior Technical Assistant in place of Shri T. V. Darnule who resigned. Shri R. J. Turel, Junior Scientific Officer, the only officer in the Raw Materials Section resigned on 10th January, 1972 and his post remained vacant.

Two posts of Senior Scientific Officer, Class I were sanctioned by Government for this department, but were not filled up.

The Department imparted training in drug analysis to candidates sponsored W. H. O., private firms, Government Institutions, private post-graduate and M. L. T. students. The following candidates were trained during period under report :—

Organisation			Total
World Health Organisation	..	..	1
Private firms	..	..	4
Private Candidates	..	..	17
M.L.T. Students	..	..	6
Grand Total			28

The number of different tests and samples analysed by the department the period under report are tabulated below :—

TABLE

Products			Tests	Number of samples tested
Bacterial vaccines	..	..	Sterility	14,372
Antirabic vaccines	..	..	"	7,937
Parenteral bulk solutions	..	..	"	8,009
Antitoxins, sera etc.	..	..	"	11,265
Miscellaneous	..	..	"	654
Parenteral bulk solutions	..	..	Pyrogen	1,217
			Chemical and physical examination.	
Parenteral bulk solutions	..	..	"	1,214
Vitamin Preparations	..	..	"	310
Sulpha drugs	..	..	"	181
Miscellaneous	..	..	"	138
Antitoxins, toxoids, vaccines, etc.	..	..	Toxicity	679

Products			Tests	Number of samples tested
Viral vaccines	..	..	.. Absence of Infective virus	311
Antitoxins	..	..	.. Potency	83
Antivenine	..	..	.. "	32
Toxoids	..	..	.. "	27
Triple Antigen	..	..	.. "	2
Vaccinos, antitoxins	..	..	Preservatives	681
Bacterial vaccines, toxoids	..	..	Free Formaldehyde	194
Pemon	..	..	.. Microbiological Assays	91
Vitamin Preparations	..	..	.. "	118
Vitamins	..	..	.. Raw Materials	65
Sulpha Drugs	..	..	.. "	72
Miscellaneous (fine chemicals, antiseptics, pharmaceutical necessities, etc.)				678
			Total	48,330

DEPARTMENT OF BACTERIOLOGY

During the year under review, the departmental activities increased manifold. Heavy demands for cholera vaccine poured in from the States of Bengal and Bihar as a result of outbreak of severe cholera epidemics in these States due to Bangla Desh refugees' concentration. Additional demands from the Director-General of Health Services and the military authorities were also met, apart from the usual demands from the State of Maharashtra.

The year brought unexpected demands for plague vaccine from the Southern States and Maharashtra. In order to meet the immediate demands for plague vaccine, a new B. P. plague vaccine, which was never manufactured at this Institute, was produced. The new vaccine was manufactured for filling up the time gap required for the production of I. P. plague vaccine and also to reach it at the site of suspected plague epidemic areas without delay. The licence for the manufacture of this new vaccine was also procured.

The production of T. A. B. vaccine was normal.

During the year, the department had to overcome several difficulties, of Roux bottles was an acute problem, as Messrs. Borocil Corning works, the only suppliers, stopped working. Besides, heavy and sudden for plague and cholera vaccines as stated above and the national increased the problems of the department. Black-out and shortage Roux hampered the production work. However, the department to turn the tide with the maximum efforts and full co-operation of staff by changing the work schedule and increasing the working hours. from the Research Unit had to be pooled for production purposes during difficult period.

Due to heavy work load and demands for cholera and plague vaccine, normal balance stock of cholera vaccine could not be kept up. The period used for stock piling was utilised for preparing plague vaccine.

Some additions and alterations were carried out in the production area suggestions from the Commissioner, Food and Drugs Administration. room was renovated with aluminium and glass partitions and ante-rooms were provided. The pooling and sterility area was also renovated and partitioned into three independent rooms, so that these could be used for test, balloon preparation and pooling of culture after harvesting. These rooms were also provided with anterooms with suitable hatches for the transfer of sterile material and other articles.

During the year, following quantities of vaccines were produced and supplied :—

Product	Produced (litres)	Supplied (litres)
Cholera Vaccine, I. P.	9,293.2	9,505.4
T. A. B. Vaccine, I. P.	557.5	105.4
Plague Vaccine, I. P.	551.7	494.8

Shri M. E. Kulkarni joined the department as Assistant Director in July 1971, in place of Shri Y. S. Nimbkar appointed as Deputy Director (Production). Kunt. J. M. Kikani was awarded the post-graduate degree of M.Sc. for her research work on "Studies in Preservation of Bacterial Cultures".

During the year under review, ten persons, including M. L. T. students and short-term trainees, were trained in the production of different types of bacterial vaccines.

MEDIA SECTION

The section continued the manufacture of culture media, stains and reagents required for the diagnosis of diseases and testing of drugs.

The manufacture of parenteral solutions also continued in full swing.

In addition to the above, the section provided general facilities for sterilization, cleaning of glassware etc. The ampoule Cutting Section supplied cut and sterilized ampoules to the Departments of Bacteriology, Immunology, Anti-rabic Section and other production departments. The production figures of the different items are presented below :—

Products	Quantity (litres)
<i>Parenteral solutions—</i>	
Dextrose and sodium chloride injections, U.S.P.	47,466·5
Dextrose injection, I.P., 5 per cent W/V	50,651·4
Sodium chloride injection, I. P.	15,817·0
Water for injection, I.P.	3,944·4
Dextrose injection, I.P., 25 per cent.	183·6
Sodium lactate, B.P.	1,970·6
A.C.D. solution formula A, U.S.P.	346·6
<i>Culture Media—</i>	
Casein hydrolysate medium (Cholera)	30,629·4
Meat-digest agar (Typhoid)	1,766·8
Casein hydrolysate agar (Plague)	718·2
Casein hydrolysate medium (Plague)	1,029·0
<i>Sterility Test Media—</i>	
Aerobic medium	7,741·4
Anaerobic medium	8,196·4
Sabouraud medium	4,518·7

The other various media and stains and solutions, which were prepared during the year, are given below :—

Products						Quantity (litres)
Acid agar	..	..	..	..	..	793·0
Nutrient agar	..	..	..	..	..	1,195·0
MacConkey agar	..	..	..	..	..	261·0
Sabouraud's agar	..	..	..	..	..	189·0
Dysentery enteric cholera agar	..	..	..	..	..	20·0
Löffler's serum medium	..	..	..	..	..	2228 test tubes × 10 ml.
Lowenstein Jensen's medium	..	..	..	..	..	1813 test tubes × 10 ml. (Litres)
Nutrient broth	..	..	..	..	..	248·0
Glucose broth	..	..	..	..	..	71·0
Peptone water	..	..	..	..	..	239·0
Glucose phosphate	..	..	..	..	..	5·5
Koser's citrate medium	..	..	..	..	..	2·5
Lab-Lemco broth	..	..	..	..	..	23·0
Thioglycolate medium	..	..	..	..	..	56·0
Ox-bile medium	..	..	..	..	..	30·7
Heartley's broth	..	..	..	..	..	8·0
Todal and Hewitt broth	..	..	..	..	..	9·0
Christensen's medium	..	..	..	..	..	6·5
Bordet Gengou medium	..	..	..	..	..	4·0
MacConkey broth	..	..	..	..	..	31·0
Sabouraud's broth	..	..	..	..	..	44·0
Alkaline peptone water	..	..	..	..	..	14·0
Selenite broth	..	..	..	..	..	1·5
Muller and Hinton medium	..	..	..	..	..	6·0
Hoyle's base	..	..	..	..	..	6·0
Buffer saline	..	..	..	..	..	17,139·0
Normal saline	..	..	..	..	..	11,746·0

Products						Quantity (litres)
<i>Sugars for biochemical tests—</i>						
Glucose	..	..	..	..	..	20·8
Lactose	..	..	..	..	..	21·6
Mannitol	..	..	..	..	..	19·8
Sucrose	..	..	..	..	..	21·3
Dulcitol	..	..	..	..	..	16·2
Xylose	..	..	..	..	..	1·1
Maltose	..	..	..	..	..	5·4
Mannose	..	..	..	..	..	0·5
Arabinose	..	..	..	..	..	0·5
<i>Diagnostic Reagents—</i>						
Ziehl-Neelsen carbol fuchsin		..	..	..	..	166·0
Albert's stain No. 1	..	..	..	..	..	18·0
Albert's stain No. 2	..	..	..	..	..	28·0
Benedict's solution	..	..	..	..	..	1,171·0
Saffranine	..	..	..	..	..	30·0
Methylenc blue	..	..	..	..	..	123·0
J.S.B. stain No. 1	..	..	..	..	..	31·0
J.S.B. stain No. 2	..	..	..	..	..	17·0
Löffler's methylene blue	..	..	..	..	..	12·0
Gentian violet	..	..	..	..	..	31·0
Preservative fluid for W.B.C.		..	..	..	..	3·0
Preservative fluid for R.B.C.		..	..	..	..	2·0
Gram's stain No. 1	..	..	..	..	..	32·5
Gram's stain No. 2	..	..	..	..	..	33·3
5 per cent Carbolic acid	..	..	..	..	..	37·5
Basic fuchsin	..	..	..	..	..	7·0

DEPARTMENT OF BIOCHEMISTRY

General

With the shifting of the Nutrition Department to the Public Health Institute, Nagpur, under the administrative control of the Director of Health Services in December 1970, the existing laboratories were rearranged on the first floor. During the year under review, the department continued its research, routine and training activities. The service facilities are particularly useful to the Employees' State Insurance Scheme and the Podar Ayurvedic Hospitals. Re-agents for clinical work were supplied to interested parties at nominal rates.

Staff

Dr. (Smt.) S. M. Modak, Senior Scientific Officer (II) went to the United States on study leave. Shri J. K. Kamath, Senior Technical Assistant, continued to officiate as Junior Scientific Officer.

Kumari Ranjana Buit, a Diamond Jubilee Research Fellow is engaged in research work on "Biochemical Studies on *V. cholerae* with reference to the action of furazolidone." Shri V. D. Rane, Junior Research Fellow, has been continued in the research scheme "Dietary fats and lipid metabolism". Shri Sayyad Raziuddin, Junior Research Fellow in this department, has taken up work on "The isolation and characterisation of proteins and ribonucleic acids for the treated and untreated cells of *V. Cholerae*".

Academic

Smt. H. V. Phadnis and Smt. Laxmi Jayaram have submitted their synopsis for the M.Sc. degree of the Bombay University. Shri U. J. Dumaswala, Senior Technical Assistant, who completed his M.Sc. degree, is continuing research work on "Lipid metabolism with reference to fats and carbohydrate components of the diet." He has registered for the Ph.D. degree of the Bombay University.

Training facilities were continued for short-term trainees in addition to the D.M.L.T. candidates of the Bombay University.

Shri J. K. Kamath, Junior Scientific Officer, attended the Pediatric Conference at Agra and read a paper in the proceedings of the conference.

Routine

A total of 4,419 tests were carried out during the year as shown below :—

Name of the test							Number of tests
<i>Body fluid :</i>							
Blood	..	..	..	..	..	..	2,920
Urine and stools	..	..	..	..	..	..	219
C.S.F. and other body fluids	..	..	..	..	..	..	93
<i>Special tests :</i>							
Basal Metabolic Rate (B.M.R.)	..	..	..	..	..	..	14
Glucose Tolerance Test (G.T.T.)	..	..	..	..	..	..	49
Cerebro-spinal fluid (Lange's colloidal gold curve)	..	..	..	..	..	..	14
Serum potassium	..	..	..	..	..	..	53
Serum protein-bound iodine (P.B.I.)	..	..	..	..	..	..	17
Serum sodium	..	..	..	..	..	..	67
Urinary 17-Ketosteroids	..	..	..	..	..	..	53
Serum glutamic oxaloacetic transaminase (S.G.O.T.)	..	..	..	..	..	..	434
Serum glutamic pyruvic transaminase (S.G.P.T.)	..	..	..	..	..	..	449
Serum phylloerythrin	..	..	..	..	..	..	6
Kidney function test	..	..	..	..	..	..	3
Serum proteins by paper electrophoresis (fractionation)	..	..	..	..	..	..	13
Gastric analysis	..	..	..	..	..	..	5
5-hydroxyindole-3-acetic acid in urine (screening test)	..	..	..	..	..	..	1
Serum phospholipids	..	..	..	..	..	..	2
Urinary stone	..	..	..	..	..	..	4
Serum ion	..	..	..	..	..	..	2
Fat in faeces	..	..	..	..	..	..	1
Total							4,419

DEPARTMENT OF CHEMOTHERAPY

During the year under review, the department continued research problems concerning the synthesis and study of newer compounds for their possible use against mycobacteria, protozoal infections like amoebiasis and filariasis, and also against neoplastic diseases. Over 260 new compounds were synthesised, characterised and studied for possible activity. Some of the compounds synthesised in the earlier period and which showed interesting activities were required to be studied for detailed pharmacological and toxicological evaluation. For this purpose, suitable methods were worked out and adequate quantities were prepared and subjected to detailed evaluations.

Some of the compounds, which had earlier shown confirmed activities in 3LE Lymphoid Leukemia, were required in further quantities by the Cancer Chemotherapy National Service Centre for tests against another tumor system, viz.: P 388 Lymphocytic leukemia (PS), recently introduced by them as having better clinically predictable potentiality. Accordingly, further quantities of these were also prepared and sent. The results have started coming in and many of the compounds have also shown not only confirmed activity of higher order but a few have registered 'cures' in these tests.

One of the thiocarbanilide derivatives, synthesised and studied for *in vitro* anti-tubercular activity in the earlier years and having shown several times more activity than the known and clinically used thiocarbanilide derivatives in leprosy was prepared in larger quantities for further studies. Its oral acute toxicity in mice has also been found to be of a much lower order.

R-1230, a piperazine derivative earlier synthesised by us and shown to possess potent hypotensive property, has been studied in detail. It is active orally and parenterally in experimental animals and acts centrally, though it does produce some peripheral action. Its main activity is due to its effect on vasomotor centers. The compound has, therefore, been prepared in larger quantity for toxicological studies.

Similarly R-585, another compound which has shown potent blood sugar lowering activity and long duration of action, was taken up for preparation in larger quantities. Suitable methods were worked out and adequate quantities were prepared and subjected to detailed toxicological studies in two different species viz. rats and monkeys.

During the year under review, Shri D. T. Chaudhari, M.Sc. and Shri K. Y. Mahajan, M.Sc., Government of India Scholars and Shri B. G. Khadse, M.Sc., University Grants Commission Fellow, continued to work on their research problems for Ph.D. degree. Shri D. R. Shekawat, Unichem Scholar, working

for doctorate degree, submitted his thesis and was awarded the Ph.D. degree. Shri M. T. Jaokar, Junior Scientific Officer in the department, completed his research problem and was awarded the degree of Master of Science.

Four research papers were published in the Journal of Medicinal Chemistry of the American Chemical Society, while one has been accepted for publication by the Indian Journal of Chemistry.

The Micro-analytical Section continued the elemental analysis of all the compounds synthesised in the department.

The production and supply of all the products manufactured by the department for the use of Government hospitals, E. S. I. S. and Public Health Bodies was continued. The output was slightly higher than that for the previous year. The list of items and their production are given in the following table :—

Name of the product						Number of tablets manufactured
Vitamin A. D. (P)	..	..	..	..	..	86,29,000
Vitamin B. C.	..	..	..	..	..	1,84,87,700
Vitamin B ₁	..	..	..	..	..	14,50,500
Vitamin B ₂	..	..	..	..	..	1,94,900
Vitamin B ₂ (Mild)	..	..	..	..	..	2,87,784
Vitamin C	..	..	..	..	..	1,23,10,000
Vitamin C (100 mg)	..	..	..	..	..	17,58,666
Nicotinic Acid	..	..	..	..	..	5,85,880
Vitamin BC	..	..	..	..	..	10,04,200
Polyvitamin	..	..	..	..	..	1,12,73,400
Vitamin B-Complex	..	..	..	..	..	1,24,72,800
Vitamin K	..	..	..	..	..	87,600
Calcium with Vitamin D	..	..	..	..	..	18,71,100
Isoniazid	..	..	..	..	..	69,37,000
Thiacetazone and Isoniazid	..	..	..	..	..	26,80,000
Iodochlorohydroxyquinoline	..	..	..	..	..	15,42,800
Di-iodohydroxyquinoline	..	..	..	..	..	20,86,100

Name of the product						Number of tablets manufactured
Sulphadiazine	..	..	..	..	..	52,36,000
Sodium aminosalicylate	..	..	..	..	..	72,10,300
Sulphadimidine	..	..	..	..	..	1,61,44,500
Sulphaguanidine	..	..	..	..	..	48,27,000
Tolbutamide	..	..	..	..	..	7,71,700
Aspirin	..	..	..	..	..	3,57,700
Chloropropamide (mild)	..	..	..	..	..	85,700
Chloropropamide (strong)	..	..	..	..	..	99,000
Phthalyl Sulphathiazole	..	..	..	..	..	11,59,600
Vitamin A. D. (Strong) E. S. I. S.	..	..	..	..	..	18,61,600
Berberine HCl	..	..	..	..	..	2,14,100
Total						12,21,26,680
Pemon (Anti-septic)	..	..	..	..	..	34,762 (litres.)

DEPARTMENT OF CLINICAL PATHOLOGY

Dr. M. V. Sant, Deputy Director (Research), handed over the additional charge of the Clinical Pathology Department to Dr. (Smt.) J. K. Dave on 2nd February 1972.

Dr. (Smt.) V. S. Vaidya, Medical Officer, Class III, Typhoid Unit, was relieved on 7th December 1971 after handing over the charge to Dr. N. B. Nadkarni. Dr. N. B. Nadkarni continued to work in the Department of Blood Bank during the emergency period till March 1972.

Shri K. K. Das was promoted as Junior Scientific Officer in the Diagnostic Reagents Section. Shri A. S. Chavan, Senior Technical Assistant of the Diagnostic Reagents Section was promoted as Junior Scientific Officer and transferred to the Media Section.

The department participated in the Inter-laboratory Evaluation Programme conducted by the Central V. D. Reference Laboratory, Madras Medical College, Madras, for the V. D. R. L. syphilis serology programme. One hundred sera were referred to this laboratory for the evaluation.

W. H. O. Combined Course in Epidemiology was conducted by this department on 27th and 28th December 1971. Discussions on various research problems of communicable diseases were arranged with the help of different departments of the Institute.

The department arranged four practical demonstrations in Clinical Pathology for the students of Diploma in General Practice referred by the College of Physicians and Surgeons of Bombay. It extended co-operations to the various other departments of the Institute in the collection of blood samples. Routine clinical pathological investigations for the entire staff of the Institute are carried out. Histopathological study of all the tissues of experimental animals received from various departments of the Institute was undertaken.

A lecture, followed by practical demonstration on Antibiotic Sensitivity Test, was held by Dr. (Smt.) J. K. Dave for the post-graduate students of M.D. in Pathology and Bacteriology.

Dr. (Smt.) J. K. Dave participated in the panel discussion on immunological test in the detection of Chorionic Gonadotrophin hormone at the Institute of Research in Reproduction, Bombay.

Twelve students were admitted for D. M. L. T. Course this year and out of these, eleven continued the course. Thirty short-term trainee students completed a short-term training course in clinical pathology.

Kumari G. V. Shirgaokar was awarded M.Sc. degree in Microbiology for research on "Toxigeny, Phage typing and Other studies on *Corynebacterium diphtheriae*".

Shri K. N. Joishy was awarded the *Gharpure Prize* for the best paper read in the Teaching Pathologists' meeting during the year 1971.

Shri K. N. Joishy submitted the synopsis of his Ph.D. thesis on the subject of bacterial genetics of mycobacterium.

Routine investigations carried out during the year 1971-72 are presented below :—

	Total
<i>A. Histopathology—</i>	
1. Histopathological examination	6,088
<i>B. Haematology—</i>	
1. Haemoglobin estimation	425
2. R. B. C. Count	405
3. Total W. B. C. Count	444
4. Differential W. B. C. Count	446
5. P. C. V. Estimation	4
6. E. S. R. Estimation	70
7. Prothrombin time	1
8. Platelet count	5
9. Bleeding Time	4
10. Clotting Time	4
11. Blood smear for Malaria	1
<i>C. Clinical Microscopy—</i>	
1. Urine for routine examination	428
2. Stool for routine examination	181
3. Semen examination	10
4. C. S. F. for Cytological examination	32
5. Other puncture fluid for cytological examination	6
6. Pregnancy Test (Qualitative)	2,376
7. Pregnancy Test (Quantitative)	256
<i>A. Bacteriology—</i>	
1. Blood for cultural examination	683
2. Urine for cultural examination	163
3. Stool for cultural examination	2
4. Throat swab for cultural examination	3
5. Antibiotic sensitivity	117
6. Autovaccine	19
7. Culture of specimens for Tuberculosis	44
8. Specimens for Guinea-pig inoculation for T. B.	13
9. Smear for A. F. B.	21
10. Culture for Diphtheria	263
11. Material for Tetanus cultural examination	175
12. Tissue for Autolysate preparation	29
13. Stool for cholera cultural examination	304
14. Smear examination for Gonorrhoea	5
15. Cultures for gonorrhoea	17
16. Miscellaneous cultural examinations	125

Total

B. Mycology—

1. Culture for fungus	1
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C. Serology—

1. Widal Test	613
2. Weil Felix Test	613
3. Test for Brucellosis	613
4. Viagglutination Test	2
5. Paul Bunnel Test	37
6. V. D. R. L. Test	496
7. Kahn Test	282
8. Wassermann Test	48
9. Casoni's (Skin) Test	3

Diagnostic Reagents Section—

1. Rideal Walker Coefficient	37
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Supply of Reagents from 1st April 1971 to 31st March 1972

	Supply (ml.)
Dead emulsion	4,61,150
High-titre sera	5,358
Kahn antigen	11,720
W. R. antigen	1,880
Casoni's antigen	506
Cobra venom solution	472
R. V. venom solution	467
<i>E. coli</i> sera	694
Vi Typhi sera	93
Vi antigen	730
Biological stains	17,98,500

DEPARTMENT OF IMMUNOLOGY

During the year, the department produced and supplied various antisera and toxoids.

There were heavy demands for tetanus and diphtheria antisera in the month of December owing to the national emergency and the department worked for extra hours to meet all the demands, especially those from the Armed Forces. The demands for antisera showed decline in the months of February and March.

There was a huge demand for 1,60,000 doses of diphtheria tetanus vaccine from the Bombay Municipal Corporation. This was supplied in full.

At the Pimpri farm, the construction of two new stables, which was started last year, was nearly completed at the end of the year.

Smt. R. V. Jaykar, Senior Technical Assistant, resigned. Shri A. D. D'Souza joined in her place. Due to financial stringency, many vacant posts in the research and production sections were kept vacant.

The following are some of the highlights of the research work carried out during the year : Drugs, such as barbiturates, chlorpromazine, paraldehyde, are used in the treatment of tetanus to reduce the spasms. It was, therefore, of interest to screen some drugs in experimental tetanus in mice to see if any of them showed anti-tetanus activity. So far, a combination of equal amounts of neostigmine and atropine in a dose of 0.025 microgram/per mouse in one dose at the time of injection of a lethal dose of tetanus toxin showed the best protective effect. It could protect against $1\frac{1}{2}$ lethal doses of tetanus toxin. The protective effect of the drugs was evident when they were injected up to 4 hours after injection of lethal dose of the toxin. This work is continuing.

The work on diphtheria phages showed that the PW8 strain used in the production of diphtheria toxin, which was considered defectively lysogenic or non-lysogenic, was found to harbour a phage when the indicator strain *C. ulcerans* was used. The PW8 strains could also be lysogenised with phage No. 3 obtained from Roumania. There was no decrease in toxin production when PW8 strain was lysogenised with phage 3.

Preliminary work done on the use of steroid hormones in normal and gonadectomised male and female mice under immunization with sheep R.B.C. that ethinyloestradiol was a powerful immuno-suppressor in gonadectomised female mice but not in normal female and male mice or gonadectomised mice.

Production—

Name of Product						Quantity (litres)
<i>Tetanus toxin :</i>						
Peptic-digest medium	..	..	..	..	..	865
Protein-free medium	..	..	..	..	..	2390
<i>Tetanus toxoid :</i>						
Peptic-digest medium	..	..	..	..	..	162
Protein-free medium	..	..	..	..	..	2256
<i>Diphtheria toxin :</i>						
Papain-digest medium	..	..	..	..	..	210
Protein-free medium	..	..	..	..	..	370
<i>Diphtheria toxoid :</i>						
Papain-digest medium	..	..	..	..	..	160
Protein-free medium	..	..	..	..	..	270
Adsorbed tetanus toxoid	..	..	..	..	..	60
Diphtheria-tetanus toxoid	..	..	..	..	..	200
Adsorbed diphtheria toxoid	..	..	..	..	..	20
Antivenin	..	..	..	..	..	161.5
Tetanus antitoxin	..	..	..	..	..	1619 M.U.
Diphtheria antitoxin	..	..	..	..	..	200 M.U.

DEPARTMENT OF IMMUNO-HAEMATOLOGY

During the year under review, the routine production activities of the department, viz. collection of blood both at the institute and the mobile processing and lyophilisation of plasma, fractionation of human plasma the production of blood grouping reagents were continued. With the tion of the national emergency, manifold expansion of the activities of department had to be organised immediately. There was tremendous spontaneous response for voluntary blood donations from the general and a large number of mobile sessions had to be arranged at different places collection of blood. There were many such mobile units organised in the and the State. All the blood collected by these units was forwarded to department for processing into dry plasma. Hence, there was

of work on this department. It was heartening to note that all the of the staff of this department as well as other staff deputed to work this department rose to the occasion and shouldered this responsibility. Ali Yawar Jung visited the department during the emergency.

of Institutions/Colleges and Factories, etc. contacted	88
of Institutions/Colleges, Factories, etc. where blood collection sessions were held.	71
of voluntary donors enrolled	8,918
<i>donors :</i>	
Voluntary	7,494
On payment	2,651
Prisoners	2,127
Hospitals	30,560
Grand Total ..	42,832

Plasma—

Name of product	Packing (ml.)	Supplied
Dried plasma	250	3,157 bottles
Human gamma globulin	1	3,937 ampoules
Human fibrinogen	150	224 bottles
Human fibrinogen	10	196 ampoules
Fresh plasma	250	15 bottles
Cryoprecipitate	100	16 bottles
Human serum albumin (in powder form)	..	95 grams
Packed cells	150	491 bottles

carried out—

Rh tests	89
Coomb's tests	7
Crossmatching tests	4
Number of anti-venine ampoules lyophilized	49,591

Blood grouping reagents—

Name of product					Packing (ml.)	Supplied
Anti-A	..	..	..	..	1	14,584 ampoules
Anti-A	..	..	..	..	5	4,632 ampoules
Anti-B	..	..	..	..	1	15,701 ampoules
Anti-B	..	..	..	..	5	4,396 ampoules
Anti-A + B	..	..	..	..	1	634 ampoules
Anti-A + B	..	..	..	..	5	262 ampoules
Coomb's reagent	..	..	..	..	1	1,050 ampoules
Bovine albumin..	..	..	..	..	1	1,715 ampoules
Anti-D	..	..	..	..	1	4,864 ampoules
Anti-A ₁ (Lectin)	..	..	..	..	1	87 ampoules
Anti-A ₁ (Serum)	..	..	..	..	1	50 ampoules
Anti-H (Lectin)	..	..	..	..	1	50 ampoules

Staff Activities

Dr. N. G. Chanderkar, M.M.S., Class II, Medical Officer In-Charge, Mobile Blood Collecting Unit, was transferred to the Department of Zoonosis from 1st August 1971.

Dr. N. B. Nadkarni, M.M.S., Class III, was posted to this Department as Medical Officer, M.M.S. Class II, Mobile Blood Collecting Unit, from 1st August 1971.

Shri Anil Muthe was appointed as Junior Scientific Officer from 18th December 1971 and Shri Ramani Nagarajan, Junior Scientific Officer, who was holding this post temporarily, also continued to work in the department in view of the heavy load of work till the end of the year.

DEPARTMENT OF PHARMACOLOGY

During the year under review, the department missed the valuable services of Shri K. G. Anantanarayanan, Senior Scientific Officer, Class I, who retired with effect from 30th November 1971, after reaching the age of superannuation and Shri V. Y. More, Senior Scientific Officer, Class II, who retired with effect from 1st February 1972, voluntarily after attaining the age of 50.

During the year under review, the following members of the staff from Microbiology Section were transferred to the Drugs Control Laboratory with effect from 1st March 1972 :—

Shri J. K. Pawoolkar, Junior Scientific Officer.

Smt. D. S. Ullengal, Junior Scientific Officer.

Shri A. D. Kothari, Senior Technical Assistant.

Smt. S. R. Pinge, Senior Technical Assistant.

Shri D. Y. Bhetasiwalla, Senior Technical Assistant.

Shri P. C. Ghosalkar, Laboratory Assistant.

Shri D. M. Mohite, Laboratory Attendant.

Shri J. V. Thakur, Laboratory Attendant.

Smt. S. R. Makhija, Typist-Clerk.

Shri A. V. Gaonkar and Shri S. B. Khandekar, Laboratory Assistants, left the department for better prospects elsewhere.

Smt. S. K. Bhinde and Kum. S. E. Bhagwagar continued as Unichem Research Fellows.

During the year under review, as many as 21 persons from various pharmaceutical concerns and private individuals were trained and given opportunity to acquaint themselves with the different techniques of analysis of drugs.

The Testing Section of the department made substantial progress during the last 3 years. The income from testing of commercial samples has reached 2 lacs, while it was merely 95,000 in 1968. Over 2,000 samples were tested during the year as compared to those in 1962. The total number of samples tested was 4,388.

During the year under review, the department continued the testing of drugs under the Drugs and Cosmetics Act and testing of drugs on behalf of Prohibition and Excise Department under the Prohibition Act, 1945. It also continued to extend testing facilities to private agencies and pharmaceutical manufacturers. Collaborative assay of phenoxymethyl potassium salt was under taken and completed. The results were submitted to the Director, Central Drugs Laboratory, Calcutta.

It is the experience of the pharmaceutical firms that they face many difficulties in analysing the formulations which contain Vitamin D₂. Many of the manufacturing firms were marketing their finished preparations without testing their D₂ content. The method described in the B. P. was partially modified to increase the recovery of Vitamin D₂ from the formulations.

Haffkine Institute is the only testing laboratory in this part of the country for testing polymorph A content in chloramphenicol palmitate suspensions. As many as 820 samples received from all over the country were tested and reported upon.

Government Analysts' Section

The Haffkine Institute Reorganisation Committee has recommended that the Government Analysts' Section, stationed at this department, should be transferred to a laboratory to be established under the then Drugs Controller, Maharashtra State. In implementing this policy, a laboratory under the Drugs Control Administration was established in 1968 with the Pharmaceutical Chemistry Section transferred from this Institute. After establishing a spacious laboratory at Bandra, the second section, dealing with microbiological testing of drugs, was transferred in March 1972. It is expected that the remaining section, i.e., the Biological Section, will also be transferred during the next year.

The total number of samples reported upon under the Drugs Act were 774, out of which 11.25 per cent of the samples were found to be non-standard.

The staff of the department also served as Government Analyst for the State of Gujarat and received only one sample during the year under review.

Many samples of phenyle, under different names, were tested for phenol coefficient and the percentage of coal-tar-acids, coal-tar oils and phenolic compounds. In a majority of the samples, phenol coefficient was less than 1 and percentage of coal-tar-acids, coal-tar oils and phenol compounds was found to be less than 15.

Nearly 200 samples of infusions, such as glucose saline, normal saline and distilled water, were tested for sterility and pyrogens. A few samples showed fungus growth and suspended particles.

Samples received from commercial concerns and Government Agencies

The commercial concerns and Government Agencies continued to take advantage of the facilities for testing of their products, amongst which were antibiotics, uterotropics, enzymes, hormones, antitoxins, toxoids for testing toxicity, potency, sterility and other factors. The demand for testing of drugs for acute toxicity and repeated dose toxicity was on the increase.

Samples received from Police and Prohibition Departments

In order to have a quality control check on toddy, samples drawn periodically by the authorities of the Prohibition Department were analysed in the laboratory for their quality and for detecting addition of harmful adulterants. One thousand thirty-two samples of toddy were tested during the year under review. In 16 samples, chloral hydrate and chloroform were detected.

For similar purposes, hard liquors, which are manufactured in the State, were received for testing. One hundred twenty-five samples were tested under this category.

Samples of narcotics, including Ganja, Bhang and Opium were tested and reported upon.

DEPARTMENT OF VIRAL VACCINES

Laboratories for production of Oral Polio Vaccine are now ready. Pilot runs have begun. Seed virus for manufacture of indigenous oral polio vaccine will be produced in Bonnet monkeys (*Maccaca radiata*) according to international regulations. This will be tested at the Haffkine Institute and at the Division of Biologic Standards at the National Institute of Health, U.S.A. Production of batches of vaccine will begin after obtaining the clearance from the N. I. H.

Processing of Oral Polio Vaccine

Pending indigenous production of oral polio vaccine, Haffkine Institute imports bulk concentrates of polio vaccine from abroad. This bulk is further diluted, tested and distributed within the country. During April 1971 to March 1972, three million doses of trivalent vaccine were distributed. In Table 1 are presented figures of distribution of oral polio vaccine for the period January 1969 to March 1972.

TABLE 1

*Figures of distribution of oral polio vaccine for the period
January 1969 to March 1972*

Month	1969	1970	1971	1972
.. ..	112715*	118570	276335	297550
.. ..	90885	120065	156440	214285
.. ..	87820	107180	126580	259640
.. ..	101730	113575	168415	
.. ..	76580	82040	170815	
.. ..	65075	107430	209020	
.. ..	79550	121280	210860	
.. ..	109185	134700	240720	
.. ..	106397	149375	252190	
.. ..	91890	245275	301580	
.. ..	101600	153625	259090	
.. ..	106900	124200	274030	
Total ..	1130327	1577315	2046035	771475

* Denotes number of doses.

DEPARTMENT OF VIROLOGY

General

Smt. Naseema A. Mehta, Diamond Jubilee Junior Research Fellow, working in this department, was awarded the Ph.D. degree of the University of Bombay in April 1971 for her studies on seroepidemiology of measles. She was appointed as a Diamond Jubilee Senior Research Fellow and continued her work in the department.

Dr. Zachariah Mathew, working on a Bhabha Atomic Research Centre grant, obtained his Ph.D. degree in January 1972 for his studies on the Rabies Virus. Dr. Mathew left this department to join his appointment as Research Officer at the Tuberculosis Chemotherapy Centre, Chetput, Madras, on completion of his Research Fellowship at this Institute.

Routine

Diagnostic studies.—The following table shows the brain samples received for rabies diagnosis during the year with results as indicated therein :—

Diagnosis of rabies during 1971-72

Animal		Number of brains received	Smear	+ve	Smear	—ve	Smear	—ve
			mouse inoculation	+ve	mouse inoculation	+ve	mouse inoculation	+ve
Dog		7*	2		1		3	
Man		2	..		2		..	
Cow		2	..		1		1	
Total		11	2		4		4	

* One dog brain, which was negative by smear, was still under mouse inoculation test.

No salivary gland samples were received.

Production :—

Antirabic Vaccine		..	..	3,852,784 ml.
Canine Vaccine		..	..	29,286 ml.
Rabies Anti-serum		..	..	229 ampoules

DEPARTMENT OF ZOONOSIS

Plague epidemiological studies were continued in 'A', 'D' and 'E' ward areas of Greater Bombay.

Epidemiological studies in Filariasis were continued and concluded in Thana area. The method of evaluation of skin test with FST antigen as a diagnostic measure for filarial infection was standardised.

Six trainees from the Bombay Municipal Corporation were trained in Advanced Entomology for three months. Shri A. V. Gosavi undertook a training course in Advanced Entomology for six months.

Six students of the Technicians' Training Course were trained in Medical Entomology.

Dr. (Smt.) S. R. Kankonkar continued to work as a C.S.I.R. Senior Research Fellow. Smt. J. R. Bharucha concluded her studies in Ascariasis.

Dr. N. G. Chanderkar, Medical Officer, Department of Blood Bank, joined this department as Senior Scientific Officer, Class II, in August 1971.

Shri D. M. Renapurkar was awarded Ph.D. degree in Zoology by the University of Bombay, for his thesis entitled "Some plague epidemiological studies in Maharashtra State". Shri N. E. Vad submitted his thesis for Ph.D. degree in Zoology in March 1972.

Combined Epidemiology International Course, arranged by the W.H.O., was conducted at the Haffkine Institute, Bombay, from 27th to 29th December 1971.

Dr. M. V. Sant attended the first meeting of "The Media for Microbiological Tests" Sub-committee on 7th and 8th February 1972 at New Delhi. He also attended the seminar on "Snake Bite" held at Ernakulum on 5th March 1972.

Dr. M. V. Sant continued to guide Shri D. S. Chitnis and Shri A. K. Patil for M.Sc. degree and Sarvashri R. S. Karbhari, Hussain Khaliefat and K. K. Das for Ph.D. degree. Under his guidance, Smt. N. A. Mehta was awarded Ph.D. degree and Shri A. S. Chavan, Shri L. M. Rumao and Kum. G. V. Shirgaonkar obtained their M.Sc. degree.

The table below details the number of snakes and other items received from outside for identification and advice :—

TABLE 1

Material received							Number
Snakes	..	..	..	..	..	..	27
Rodents, insects and other queries			..	..	..	..	7
Rodents and insect control queries			..	..	..	..	5
					Total	..	39

Besides attending to the above inquiries, this department also supplied insects, snakes, rodents and micro-slides of insects to a number of educational and research institutions. The number of insects, snakes, rodents and slides supplied is shown in the table below :—

TABLE 2

Material supplied	Number
Dead Cobras	11
Dead <i>Russell's viper</i>	11
Dead Kraits	51
Dead <i>Echis carinata</i>	23
Dead non-poisonous snake	1
Dead Snake skins	15
Dead <i>X. cheopis</i>	5

Colonies of fleas (*X. Cheopis* and *X. astia*), flies (*M. domestica nebulo*), roaches (*P. americana*), mosquitoes (*Culex P. Fatigans*) and rodents (*indica*), were continued to be maintained for insecticide and plague

Visitors

Visitors coming to this department were shown the venom technique by actual demonstration and were given detailed information the identification of snakes and first-aid treatment of snake-bite cases, etc. The total number of visiting teams and distinguished visitors, who visited department, is shown in the following table :—

TABLE 3

Details	Number of teams and demonstrations
Students from different Colleges	9
Visitors from scientific institutions	12
Distinguished visitors	14
Regular demonstrations for visitors	33
Total	68

Production

During the period under review, 199·92 gms. of snake venoms were processed and 187·40 gms. of venoms were supplied for the manufacture of Antivenin and research purposes as shown below :—

TABLE 4

Particulars					Processed	Supplied
<i>Snake Venoms—</i>						
Krait	..	..	..	..	42·66 gms.	51·26 gms.
Cobra	..	..	..	..	110·32 gms.	73·49 gms.
Russell's Viper	..	..	..	..	37·28 gms.	44·72 gms.
Echis	..	..	..	..	9·66 gms.	17·93 gms.
Total					199·92 gms.	187·40 gms.

The following scorpion venoms were processed and supplied :—

Particulars					Processed	Supplied
<i>Scorpion venoms—</i>						
Black scorpion	..	..	..	..	1·46 gms.	Nil.
<i>Palamaneus gravimanus—</i>						
Red scorpion	..	..	..	..	1·11 gms.	1·00 gm.
<i>Buthus tamulus</i>						
Total					2·57 gms.	1·00 gm.

TABLE 5

The following snakes and scorpions were purchased during the year :—

Cobra	..	..	..	..	..	218
Krait	..	..	..	..	..	877
R. Viper	..	..	..	..	..	134
Echis	..	..	..	..	..	1871
Non-pisonous snakes	..	..	..	..	..	184
Red scorpions	..	..	..	..	..	847
Black scorpions	..	..	..	..	..	3899

ANIMAL FARM

The breeding and supply of different species of laboratory animals were satisfactory. The farm met the requirements of the departments during the year under report. As a result of the all-out strike in November 1970, guineapig colony suffered to some extent and 75 guineapigs had to be purchased from other breeding places, as production was low in the months of May, June and July. Similarly, 540 mice of Haffkine strain were purchased from Hindustan Antibiotics Ltd. for specific work of plague vaccine testing.

Students of D.M.L.T. and Technicians' Training Course were trained in the general management of laboratory animals. Trainees of the Laboratory Animals Technicians' Training Course conducted by the Laboratory Animals Information Service (I.C.M.R.) were provided facilities for practical work in connection with their course. Shri K. D. Muntode, Animal Technician from Marathwada University and Shri Desai from Surat (Gujarat State) attended the training in breeding, maintenance and management of animal house. The figures mentioned below will show the number of different species of laboratory animals bred in the Animal Farm and supplied to the departments and outside during the year under review :—

			Production	Supplied to departments	Supplied outside
White mice	..	..	2,56,684*	2,00,533	15,385
Albino rats	..	..	13,229	6,060	5,147
Rabbits	..	..	3,620	2,380	1,355
Guineapigs	..	..	5,210*	3,982	923
Hamsters	..	..	1,250	592	96
Cotton rats	..	..	59	2	

* Includes the animals procured from other laboratories (mice 540 and guineapigs 75).

In addition to the breeding of the abovementioned animal species, sheep, monkeys, cats and fowls were maintained in the Animal Farm as per the requirements of different departments of the Institute.

LIBRARY SECTION

Reorganisation

The reorganisation has been proceeding satisfactorily. The reading room for the current issues of journals has been air-conditioned and provided with better illumination. For the book section, a list of subject headings has been prepared and is kept in the form of card index for the readers' use. Also, card-indices for scientific and technical reports, theses and the research papers published from the Institute have been prepared.

During the year, 6 new journals were added to the list of journals subscribed annually. The extension of working hours to 7-00 p.m. was continued the year. The library continued to participate in the inter-library loan and also participated in the international exchange programme sponsored by the W.H.O., American Medical Library Association, British Medical Association and UNESCO.

working

The following figures indicate the working of the library during the year :—

Number of outside readers visiting the library	3,540
Number of books and periodicals issued to our staff ..	900
Number of periodicals issued under inter-library loan service.	237
Books and periodicals obtained for our staff from other libraries.	46

Additions to Library—

Number of books added during the year	278
Number of volumes bound	347
Number of current periodicals subscribed	168
Current periodicals received free of charge	100
Number of reports of scientific institutions received ..	45

of papers published during the year 1971-72

Dave (Smt.), J. K. and Sant, M. V. : Carcinoma of the breast in a girl aged fourteen (1971). *Indian Journal of Cancer*, 8, 131-133.

Deoras, P. J., Bhatt, S. M., and Dhamanaskar, C. A. : Observations on cross-breeding and rearing of a strain of Mice for plague work (1971). *Pesticide*, 5, 8-12.

Dimitrov, G. D. : Purification and partial characterization of two proteolytic enzymes from the venom of *Vipera Russellii* (1971). *Toxicon*, 9, 33-44.

Dutta, N. K. and Fernando, G. R. : Antifertility action of sodium acetate in animals (1972). *Indian Journal of Medical Research*, 60, 48-53.

Dutta, N. K., Marker (Kum), P. H. and Rao, N. R. : Berberine in toxin induced experimental cholera (1972). *British Journal of Pharmacology*, 44, 153-159.

Dutta, N. K., Nimbkar, Y. S., Karbhari, R. S., Markar (Kum.), Perviz and Kumar (Smt.), Shakuntala : Experimental studies of live cholera vaccines prepared from *V. Cholerae* C₁₄ S₅ and El-tor Water 6 (1971). *Symposium on Immunity and Immunoprophylaxis in Cholera*.

Dutta, N. K., Rao, S. S. and Chatterjee, P. K. : Antigenicity of cholera toxoid (1971). *Symposium on Immunity and Immunoprophylaxis in Cholera*, 13.

Kumar (Smt.), Shakuntala and Dutta, N. K. : An experimental study of non-agglutinable vibrios which caused "Gastro-enteritis" in clinical cases (1971). *Indian Journal of Medical Research*, 59, 1378-1384.

Meher-Honji (Smt.), Villie K. and Gharpure, P. V. : "Non-Specific" inhibitors in normal animal sera against polioviruses (1971). *Indian Journal of Medical Sciences*, 25, 231-235.

Mhasalkar, M. Y., Shah, M. H., Pilankar, P. D., Nikam, S. T., Anantanarayanan, K. G. and Deliwala, C. V. : Synthesis and hypoglycemic activity of 3-Aryl (or Pyridyl)-5-alkyl (or aryl) amino-1, 3, 4-this-diazoles and some sulfonylurea derivatives of 4H-1, 2, 4-triazoles (1971). *Journal of Medicinal Chemistry*, 14, 1000-1003.

Modak, M. J., Aroskar, V. A. and Bhide, M. B. : Observations on the Type 2 and Type 3 toxins of *Vibrio Cholerae* (1971). *Symposium on Immunity and Immunoprophylaxis in Cholera*.

Mody, J. D., Sabnis, S. S. and Deliwala, C. V. : Antitumour Agents—Schiff bases from benzaldehyde nitrogen mustards and 2-phenyl-4-[(3'-Amino-4'-Methoxy) Phenyl]-thiazole (1971). *Journal of Medicinal Chemistry*, 14, 450-451.

Modi, J. D., Sabnis, S. S. and Deliwala, C. V. : Potential anticancer agents—nitrogen mustards of aminophenyl thiazoles (1971). *Journal of Medicinal Chemistry*, 14, 887-888.

Nimbkar, Y. S., Karbhari, R. S., Rao, N. R. and Jhala, H. I. : Biological assay of cholera vaccine (1971). *Symposium on Immunity and Immunoprophylaxis in Cholera*.

Nimbkar, Y. S., Karbhari, R. S., Renapurkar, D. M. and Sant, M. V. : Observations on susceptibility status of rats from different localities in Thana District to experimental plague infection and serological studies for detection of foci of infection among domestic rodents (1971). *Pesticide*, 59, 1203-1208.

Pandit C. G. : India and the Yellow Fever Problem (1971). *Indian Journal of Medical Research*, 59, 1523-1547.

Panse, M. V. and Nayak, K. P. : Usefulness of a medicinal Plant—*Bragantia Wallichii*—in experimental cholera (1971). *Indian Journal of Medical Research*, 59, 1190-1193.

Renapurkar, D. M., Chaturvedi, G. C., Vad, N. E., Tare, T. G. and Sant, M. V. : Plague epidemiological studies in Nasik District, Maharashtra State—Part-I—Rat and flea survey (1971). *Indian Journal of Communicable Diseases*, 3, 182-189.

Rumao, L. M. and Sant, M. V. : The serological grouping of beta-hemolytic streptococci isolated in Bombay (1972). *Indian Journal of Microbiology*, 12, 31-35.

Saldanha, F. L., Jhala, H. I., Sant, M. V. and Nimbkar, Y. S. : Survival of *Vibrio cholerae* in human bile and its detection in infant rabbits gall-bladder (1972). *Indian Journal of Medical Research*, 60, 173-177.

Saldanha, F. L., Sant, M. V. and Nimbkar, Y. S. : Studies on symbiosis of *Vibrio Cholerae* (1971). *Symposium on Immunity and Immunoprophylaxis in Cholera* 174-185.

Shinde, V. P. and Ambegaokar, S. D. : Pantothonic acid distribution and protein synthesis in the particulate fractions of rat liver (1971). *Experientia*, 27, 1164-1165.

Shringi (Kum.), Sharda, Kankonkar, R. C. and Rao, S. S. : Studies on the free amino acids of the haemolymph of *Poeciloceris pictus* (Acridae) during cycle (1971). *Current Science*, 40, 563-566.

Tamhane, R. G. and Rao, S. S. : Estimation of thymol in analgesic Balm
Indian Journal of Pharmacy, 33, 70-72.

of papers sent for publication during the year 1971-72

Baxi, A. J. : Hereditary red cell, enzyme defects and hemolytic anemia
Medical Journal.

Baxi, A. J., Kulkarni, K. V., Bellare (Kum.), Jyoti A., Patankar, V. M.
Australia antigen and the liver function. *Experientia*.

Baxi, A. J., Kulkarni, K. V., Patankar, V. M. and Bellare (Kum.), Joyti A. :
antigen, hepatitis and liver function. *Indian Journal of Medical*

Bhat, A. K., Bhamaria, R. P., Patel (Smt.), M. R., Bellare, R. A. Deliwala, C. V. : Chemotherapy of fungus infections part III : Alkyl or Thiosemicarbazones, Acid Hydrazones and Styryl Aryl Ketones of -5 and—Nitrosalicyl—aldehydes. *Indian Journal of Chemistry*.

Darnule, T. V. and Anantanarayan, K. G. : Effect of caffeine on diabetic drugs (Short communication). *Indian Journal of Pharmacy*.

Das, K. K. and Sant, M. V. : Evaluation of the Immunofluorescent test in typhoid. *Journal of Post Graduate Medicine*.

Das, K. K. and Sant, M. V. : Hemagglutination test as an aid in the laboratory diagnosis of enteric fever. *Indian Journal of Pathology and Bacteriology*.

Dave (Smt.), J. K., Fernandez, Y and Sant, M. V. : Urethral Rhinosporidiosis reports of two cases with a brief review. *Indian Journal of Medical Science*.

Deoras, P. J. : Tolerance Shown by *Rattus rattus* to an anticoagulant rodenticide. *Indian Journal of Medical Research*.

Deoras, P. J. and Karnikar (Kum.), K. : Breeding of *Musca sorbens*, Wied in the laboratory. *Indian Journal of Entomology*.

Desai, S. M., Sehgal, P. B., Nanavati, A. N. D. and Shirodkar, M. V. : Rabies induced serum factor inhibits the Rous Sarcoma virus in chicks. *Nature*.

Fernandez, J. A., Bellare, R. A. and Deliwala, C. V. : Synthesis and central nervous system activity of new piperazine derivatives IV (Note). *Journal of Medicinal Chemistry*.

Hakim (Kum.), S. M. A., Baxi, A. J., Balkrishnan, V., Kulkarni, K. V., Rao, S. S. and Jhala, H. I. : Glucose-6-phosphate dehydrogenase deficiency and colour-vision studies in Indian Muslims. *Humangenetik*.

Hakim (Kum.), S. M. A., Baxi, A. J., Balkrishnan, V., Kulkarni, K. V., Rao, S. S. and Jhala, H. I. : Haptoglobin transferrin and abnormal haemoglobin in Indian Muslims. *Indian Journal of Medical Research*.

Kulkarni, K. V., Baxi, A. J. and Patankar, V. M. : Studies of Australia antigen and hepatitis. *Journal of the Indian Medical Association*.

Kulkarni, K. V., Baxi, A. J., Patankar, V. M. and Das, P. C. : Studies on Australia antigen in India. *Vox Sanguinis*.

Mathew, Z. : Study of interference against rabies virus. *Indian Journal of Medical Research*.

Mehta (Smt.), Naseema, A., Nanavati, A. N. D., Jhala, H. I. and Sant, M. V. : Seroepidemiology of measles in Bombay and measles antibodies in commercial preparations of Gamma Globulin. *Indian Journal of Medical Research*.

Rao, S. S. : Hazards of immunization. *Vigyan Pragati*.

Renapurkar, D. M., Bhat, V. M., Menon, T. U. K. and Sant, M. V. : Some observations on duration of different Stages of Life history of *Culex Pipiens Fatigans* Wied. *Current Science*.

Renapurkar, D. M., Chaturvedi, G. C., Bhat, V. M. and Sant, M. V. : Evaluation of new rodenticide MR-100 containing a taste enhancer. *Indian Journal of Experimental Biology*.

Roy, R. N., Mhasalkar, M. Y., Bhagwagar (Kum), Silloo and Dutta, N. K. : Pharmacological actions of VIDR—2 GD, —a fraction isolated from seeds of *Banakadali* (*Ensete Superbum*, Cheesm *Musaceae*). *Antiseptic*.

Rumao, L. M. and Sant, M. V. : Evaluation of Bacitracin disc for the identification of B Hemolytic Streptococci Group A. *Indian Journal of Microbiology*.

MAINTENANCE ENGINEERING AND CARPENTRY SECTION

During the year under review, the Section continued to be fully busy with increased work-load and maintenance of equipment etc.

This year, due to increased work-load and the national emergency, the engineers and technical staff of the Section carried out a number of urgent jobs departmentally. In the case of the Desivac Plant required by the Blood Bank Department, great care was taken in order to avoid any break-down during emergency. It was a pleasure to note that there was not a single break-down. In addition to this, the air-conditioning section has carried out a number of additions and alterations in the Blood Bank Department, such as repairing deep-freeze, refrigerators, sealed compressors, etc. There are a number of deep-freeze in the Polio Section and the same were maintained by the Engineering Section in order to avert the production losses.

It is gratifying to note that the mechanical section carried out a number of jobs throughout the year, such as preparation of discs for filter paper cutting as per the requirements of the Polio Section ; preparation of a device for drilling holes in monkey skull (the device was made out of stainless steel as per design brought from England) ; designing, fabrication and assembling agar bottle rolling machine for vaccine production. The job of erecting the distillation unit for the Media Section was taken up this year. In addition to this, the Maintenance Engineering Section carried out a number of turning jobs on our lathe, as per the requirements of the production and research departments. Such types of jobs, if given outside, are very costly. This has resulted in considerable financial saving as well as in the time factor. Further, new machinery erection jobs, including necessary additions and alterations, were carried out. The electrical section played a major role specially during the time of the national emergency. The staff carried out a number of electrical connections to the machinery connected with production. A larger number of heating ovens were assembled and brought into beneficial use in the Chemotherapy, Bacteriology, Polio and Zoonosis Departments. The maintenance of the automatic electric panel board attached to the boiler in the Toxin Section, which was previously entrusted to an outside agency, was taken over by this section. The distillation plant in the Immunology Department was put into beneficial use and its complete wiring was carried out. Four incubation rooms, with special type of head controlling devices were provided in the Polio Section. Further, a large number of jobs like repairing various types of ovens, centrifuges, machines, air compressors, vacuum pumps, stirrers, pharmaceutical machinery etc. were carried out. In addition to this, various types of electric motors, numbering about 1800, were looked after and maintained.

The Carpentry Section played a major role in the maintenance work of the Institute during the time of national emergency. A number of jobs like temporary structures, cabins, wooden stands for test tubes, wooden crates of different sizes for animals, additions and alterations in laboratory tables, were carried out.

In short, this Section has played a significant role in the total out-put.

