



सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

**HAFFKINE INSTITUTE
BOMBAY**

**ANNUAL REPORT
1966-67**

Printed in India by the Manager, Government Central Press, Bombay,
Published by the Director, Government Printing, Publications and
Stationery, Maharashtra State, Bombay-4

Maharashtra Government Publications can be obtained from—

THE DIRECTOR

Government Printing and Stationery,
Publications Branch, Charni Road Gardens,
Netaji Subhash Road . . . BOMBAY-4

THE SUPERVISOR

Government Book Depot,
(for Central Government Publications)
Charni Road Gardens,
Netaji Subhash Road . . . BOMBAY-4
(Sale on counter only)

THE MANAGER

Government Photozinc Press
and Book Depot POONA-1

THE MANAGER

Government Press and Book Depot
Civil Lines NAGPUR-1

THE ASSISTANT DIRECTOR

Government Stationery Stores and
Book Depot AURANGABAD

THE HIGH COMMISSIONER FOR INDIA

India House
Aldwych LONDON, W.C.-2

AND

THE RECOGNISED BOOK SELLERS

HAFFKINE INSTITUTE, BOMBAY

ANNUAL REPORT

1966-67

CONTENTS

PART I

1. General Review	..	..	..	..	3
-------------------	----	----	----	----	---

PART II

A. RESEARCH PROJECT :—

(a) Cholera Project	..	..	..	..	11
(b) Diphtheria Project	..	..	..	..	13
(c) Enterovirus Project	..	..	..	..	17
(d) Plague Project	..	..	..	..	21

B. OTHER DEPARTMENTAL RESEARCH :—

1. Department of Bacteriology	..	..	..	25
2. Department of Blood Bank	..	..	..	29
3. Department of Chemotherapy	..	..	..	33
4. Department of Clinical Pathology	..	..	..	35
5. Department of Entomology	..	..	..	38
6. Department of Immunology	..	..	..	40
7. Department of Nutrition and Biochemistry	..	..	..	46
8. Department of Pharmacology	..	..	..	52
9. Department of Virology	..	..	..	55

PART III

ROUTINE ACTIVITIES

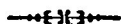
1. Analytical and Control Department	..	..	..	65
2. Department of Bacteriology	..	..	..	66
3. Department of Blood Bank	..	..	..	69
4. Department of Chemotherapy	..	..	..	71
5. Department of Clinical Pathology	..	..	..	73
6. Department of Entomology	..	..	..	75
7. Department of Immunology	..	..	..	75
Department of Nutrition and Biochemistry	..	..	..	77

PART III— <i>contd.</i>				PAGE
9.	Department of Pharmacology	..	..	82
10.	Department of Virology	..	..	85
11.	Animal House	..	..	87
12.	Library Section—			
	(a) List of papers published	..	..	88
	(b) List of papers awaiting publication	..	..	92
13.	Maintenance Engineering Workshop and Carpentry Section		..	94

PART IV

MISCELLANEOUS

1.	Critics Club	..	..	97
2.	Distinguished Visitors	..	..	99
3.	Academic Courses and Training of Health Personnel	..	..	101
4.	No. of post-graduate students on roll	..	..	102
5.	Candidates obtaining post-graduate degrees	..	..	103
6.	Short-term trainees	..	..	104
7.	Pool Officers	..	..	105
8.	Students and Trainees admitted	..	..	105
9.	Research Fellowships and Scholarships	..	..	107
10.	Guest workers and trainees	..	..	111
11.	Grants-in-aid Research Schemes	..	..	111
12.	Business Side	..	..	113
13.	Welfare Activities	..	..	117
14.	Haffkine Institute Co-operative Credit Society	..	..	118
15.	Dispensary	..	..	118



INSTITUTE, BOMBAY

MEMBERS OF THE STAFF

Director

Dr. H. I. Jhala, M.D. (Bom.), F.C.P.S. (Bom.), D.T.M. (Cal.), F.F.C. (Path.) (London).

Assistant Directors

Dr. N. K. Dutta, M.B. (Cal.), D.Phil. (Oxon.), D.Sc. (Oxon.), F.N.I., Director In-charge, Department of Pharmacology.

Dr. P. J. Deoras, M.Sc. (Nagpur), Ph.D. (Durham), F.E.S.I., LL.B., Director In-charge, Department of Entomology.

Dr. C. V. Deliwala, M.Sc. (Bom.), Ph.D. (Bom.), Assistant Director In-charge, Department of Chemotherapy.

Dr. M. V. Sant, M.D. (Bom.), F.C.P.S. (Bom.), Assistant Director Department of Clinical Pathology and Diagnostic Reagents (On from 7th November 1966).

Dr. K. H. Dave, M.D., Assistant Director In-charge, Department of Clinical Pathology and Diagnostic Reagents (from 22nd February 1967).

Dr. A. N. D. Nanavati, M. D., Assistant Director In-charge, Department of Virology.

Dr. (Smt.) Alamela Venkataraman, B.A. (Madras), M.Sc. (Madras), Ph. D. (Madras), Assistant Director In-charge, Department of Nutrition and Biochemistry.

Dr. S. S. Rao, M.Sc. (Benaras), Ph.D. (Toronto), Assistant Director In-charge, Department of Immunology.

Dr. K. V. Kulkarni, M.B.B.S. (Bom.), Assistant Director In-charge, Department of Blood Bank.

Shri Y. S. Nimbkar, M.Sc. (Bom.), Dip. Bact. (Manchester), Assistant Director In-charge, Department of Bacteriology.

Dr. M. K. Habbu, B.Sc., Ph.D. (Bom.), Assistant Director In-charge, Department of Analytical and Control.

Heads of Sections

A. E. Kulkarni, G. B. V. C. (Bom.), Senior Scientific Officer (Class I) Animal Farm.

Administrative and Auxiliary Sections

Shri Y. M. Phondge, Personal Assistant to the Director (Upto 15th May 1966).

Shri T. V. Ranganathan, B.A. (Madras), Personal Assistant to the Director (From 16th May 1966).

Shri P. K. Sholapurwala, B.Sc. (Bom.), LL.B. (Bom.), Business Manager.

Shri A. T. Maniar, B.Sc., Stores Officer.

Shri T. V. Ranganathan, B.A. (Madras), Stocks and Supplies Officer (Upto 15th May 1966).

Shri M. T. Jagtiani, B. A., Stocks and Supplies Officer (From 16th May 1966).

Shri M. T. Jagtiani, B.A., Special Officer (Recoveries)—(Additional charge upto 31st March 1967).

Shri B. A. Sakhardande, Labour Welfare Officer (Upto 1st March 1967).

Shri N. R. Rao, M.A., Statistician.

Shri S. J. Haridas, Assistant Engineer (Maintenance) (Upto 26th April 1966).

Shri M. R. Choudhari, Assistant Engineer (Maintenance) (From 18th August 1966).

Shri M. N. Manohar, B.Sc. (Poona), Dip. Lib. (Bom.), Librarian.

PART I

GENERAL REVIEW

GENERAL REVIEW

The year 1966-67 was a critical financial year for the country. The was faced with acute foreign exchange crisis resulting in devaluation of currency. The country was also faced with famine situations and economy had to be exercised in the expenditure at all levels, resulting in down of the tempo of development and expansion. While every care taken to keep the supplies going, the new construction activity remained at a stand-still, resulting in greater backlog in the construction work. market prices of raw materials did not remain stable. The prices of products could not be revised owing to the National Price Control Regulations for drugs.

During the year, the academic courses were continued as before and were fully subscribed. There were 15 recognised teachers of the University and 32 students for M.Sc., 20 students for Ph.D. and 3 students for the other post-graduate examinations. Seven students took their M.Sc. and 4 obtained Ph.D. Besides this, 12 students completed D.M.L.T. course and 9 students completed the Technicians' Training Certificate course. Two Pool of the C.S.I.R. were given placement at the Institute. Besides this, 25 short-term trainees were entertained in various departments of the Institute for specific training. The number of fellowships during the period was 36 and most them were occupied by students and post-doctoral workers. There were 5 guest workers from various countries abroad working in specific branches of their interest. During the year under review, C.S.I.R. started two new research schemes in Chemotherapy Department and the I.C.M.R. financed three research units and two enquiries. The institution also received research grant from W.H.O. for research on cholera. The total grant-in-aid for research exceeded Rs. 3.5 lakhs.

During the year under review, the project-wise orientation for interdisciplinary research was continued in the form of principal projects of cholera, diphtheria, enteroviruses and plague.

In the cholera project, two different approaches using mouse protection test and passive infant rabbit protection tests were made for standardisation of cholera vaccine. Attempts were made to evolve a better vaccine through attenuation and also through fractionation and characterization of toxin. A new chemotherapeutic agent in the form of Berberine hydrochloride has been found to be clinically effective in cholera during clinical trials carried out in collaboration with the Infectious Diseases Hospital in Calcutta, and it compares well with antibiotic therapy. Biochemical studies on the nutritional requirements of Cholera vibrios are in progress.

The work on role of phage in induction of toxin and the role of iron in the control of toxin production has been completed. Attempts are being made to convert non-toxinogenic diphtheria strain into toxinogenic strain without the

mediation of phage. Some new laboratory strains have been isolated and are under study. The role of adjuvants and hormones is being experimentally studied in regard to the yield of diphtheria antibodies.

The enterovirus project has studied the acute gastro-enteritis in children, non-vibrio cholera, aseptic meningitis and encephalitis in Nagpur. A number of isolates have been made and the serological identification is nearing completion. Titration of antibodies in paired sera against isolates will soon be done. The Echo 7 virus seems to have some predominance in the isolates. While the results as presented are interesting, further work requires to be done for assessing the roles of the isolated viruses.

The study on rodent fauna in Vidarbha was completed last year. The rodent fauna of Marathwada is being reported this time. The trial of rodenticides in the field and also trial of various techniques developed was carried out. Studies on rodent susceptibility to plague are being continued. An attempt is made to set up a standard for Casein Hydrolysate Plague Vaccine for use in bioassay.

Experiments to produce acetone-killed and acetone-dried typhoid vaccine have progressed satisfactorily. The W.H.O. Consultant from Walter Reed Army Institute of Medical Research visited on behalf of the W.H.O. to render assistance to the project. Methods are being evolved for testing the typhoid vaccines on rabbits for their efficacy.

The number of blood donors has dwindled. Efforts are being made to contact institutions. Government have sanctioned a Propaganda Unit as suggested by the State Advisory Board. All blood grouping reagents are now available. Study of genetical characters of some endogamous groups is in progress. The bone grafts supplied by the Institute are successful. Attempts are being made to render help in the supply of preserved heart valves.

The synthesis of anti-tubercular compounds, cytotoxic agents and synthetic diuretics and CNS depressants has progressed well. Studies on manganese poisoning have been completed and studies on parathion exposure have been undertaken. A number of compounds have been screened by the Drug Screening Unit for anti-bacterial and anti-fungal properties.

Studies on *Staphylococcus aureus* have been completed and studies on Leptospirosis and its epidemiology are in progress. Some incidence of leptospirosis has been seen in pigs and rats. A few cases of leptospirosis in man have also been detected. Passive hæmagglutination tests seem to be more reactive and specific than the conventional agglutination tests in typhoid fever.

Studies on the biology of *M. sorbens* are nearing completion. The feeding mechanism of fleas has been investigated fully. Zinc phosphide seems to have promising results as raticide. Cross-protection given by the Haffkine Institute Polyvalent Antivenine against King Cobra, banded krait and Bamboo Pit Viper Venoms has been noted.

The agar overlay technique for isolation of *Clostridia* has been successfully. Bacteriocine has been isolated from *Clostridia*. Russell's Venom has been fractionated and different fractions seem to differ in composition and characters. Studies on the nutritional requirements of *B.* and the nature of its protective antigen are completed. The studies on the structure of immunoglobulins in horse sera seem to be very promising. The M. R. C. trials of simultaneous active and passive immunisation against tetanus have been concluded. The scheme of study of lens protein is continued by Government and assistance has been asked for from P. L. 480 funds.

It has been possible to adopt microbiological methods for evaluation of proteins. Protein metabolism has been studied in relation to Vitamin B₁₂ and Pantothenic Acid. Nutritional requirements of *Salmonella*, *V. cholerae*, *P. pestis*, *E. coli* and *C. diphtheriae* have been studied by the department. Some studies in dietary fats of Sindhi community have been developed for transaminases.

Anti-diabetic and anti-amœbic drugs have been studied in detail. Work on isolation and characterisation of natural products of plants has been undertaken as per the programme. Anti-bacterial activities of some indigenous medicinal plants have been studied.

Studies have been carried out to titrate rabies virus in tissue culture without the use of mice. In the studies on vaccinia, it has been shown that plaque formation by virus and neutralization by antiserum in agar overlay cultures was more consistent and reproducible than pock formation in chorioallantoic membrane. Studies have been carried out on stability of polio vaccine under varying conditions. Genetic markers and non-specific inhibitors against polio were also studied. The monkey kidney tissue cultures were studied for their extraneous agents.

The Analytical and Control Department has taken over testing of almost all drugs issued from the Institute. The new building has not yet been sanctioned for this department.

The Department of Bacteriology has switched over to mixed *El-tor* and *Cholera* Vaccines as per instructions received from the D. G. H. S. Special vials were provided for use of jet gun for vaccination at Pandharpur. It is proposed to issue T. A. B. Vaccine in rubber-capped vials.

The clinical use of plasma fractions seems to be growing.

Due to non-availability of sulphathiazole and sulphadiazine, the production had to go down. Sulphadimidine has been started to replace thiazole and diazine. Sulphaguanidine is still being supplied, as there is a demand. Increased production of Pemon has been undertaken. Chlorpropamide has been marketed.

The Departments of Biochemistry and Clinical Pathology continued to render assistance in diagnostic field. The radio-isotope work, especially in relation to diagnostic work for thyroid disorders is likely to start soon.

The total amount of venom produced was about 155 gms. and the total venom supplied was 189 gms. The Institute participated in the Indian Rodent Symposium. A committee for rodent control has also been formed for the City of Bombay.

The Department of Nutrition and Biochemistry has taken over supervision of industrial canteens and participation in training of functionaries of the Applied Nutrition Programme.

The programme of drug testing continued in full swing. Out of 3,000 samples of drugs analysed, only 15 per cent were found to be of non-standard quality in the market. The percentage of non-standard preparations is not in excess of what was noted last year.

The production of antirabic vaccine has slowed down, as the uptake from Gujarat has diminished. The opening of new centres in Maharashtra has been continued, but the new centres are not picking up the vaccine in sufficient quantities. It has been decided to supply antirabic vaccine to listed practitioners by post in the same way as on sales counter. Polio Vaccine administration on premises is discontinued. A five-dose and a hundred-dose packing are available from the Institute.

The animal house continued to supply the animals. Owing to difficulty of availability of gram, oat, barley, we had to change over to Hind Lever cattle-feed for horses. For horses, there was increasing difficulty of obtaining lucerne grass owing to draught conditions. This has affected the guinea-pig colony. Due to non-availability of imported milk powder, it had to be substituted by groundnut-cake. Every effort is being made to look into the quality and quantity of output of the animals. But this has involved a great strain.

The profitability of the Institute considerably dwindled due to rise in raw material and packaging prices, rise in wages, and reduced uptake by the Government during the year. The expenditure also rose on account of devaluation. Production activity supports itself, but is unable to bring about the return which would pay for augmenting research, training and other programmes now carried out at the Institute.

Dr. V. N. Jai, S. S. O. Class I, Department of Nutrition and Biochemistry, returned after his training in London and Ibadan. Dr. (Kum.) Shanti Arora, Senior Nutritionist, went as a short-term W.H.O. Consultant to F.A.O. for a period of four months. Dr. H. I. Jhala attended the IXth International Congress of Microbiology at Moscow as a Guest of Honour, at the invitation of the national scientists of U.S.S.R. Dr. N. K. Dutta participated in the Pan-Pacific Congress at Tokyo. Dr. P. J. Deoras attended the conference on venomous animals at Sao Paulo in Brazil and the symposium on animal-keeping in Ireland. Dr. M. V. Sant proceeded on a W. H. O. Fellowship to take up advanced training in epidemiology including Zoonosis. (Kum.) M. S. Kotnis, Factory Canteen Adviser, was assigned as F.A.O. Expert on nutrition to Malawi from February 1967 for a period of 18 months.

The total number of papers published during the year was 36 in addition 29 papers sent for publication. The library grant had to be increased, as subscriptions went up due to devaluation.

There were 30 meetings of the Critics Club during the year, some of were addressed by visiting guests. A delegation of the Estimates Committee Parliament and also the Select Committee for Patent Bill visited the during the year as also a number of distinguished visitors from the country and abroad.

A committee was appointed by the Government to go into the question building programmes of the Institute with a view to find out if any activities could be shifted elsewhere. The committee visited the Vaccine Institute, Nagpur, from the viewpoint of shifting production of vaccines to the Vaccine Institute, Nagpur, in future. The report is under the consideration of the Government.

Another committee was appointed to examine whether production of non-biologicals could be separated from the other activities of the Haffkine Institute. The interim report of the committee is before the Government. The final report is still awaited.

The State Advisory Board met twice in the year and made valuable suggestions. The Haffkine Institute Research and Development Fund Committee has its half-yearly meetings.

It would, therefore, appear from the above that the research and training programmes have been well augmented. This is due to sanction of additional staff for research during the period.

The Director is grateful for the assistance received from the Government. Thanks are also due to the various research councils and other agencies for their grants. Special thanks are due to the State Advisory Board and other committees that helped the Institute during the year. The help received from the various Heads of Departments and members of the staff is gratefully acknowledged.

PART II

RESEARCH PROJECTS

A. RESEARCH PROJECTS

(a) Cholera Project

(i) *Bioassay of live cholera vaccines and their antigenic interrelationship by infant rabbit model (Department of Pharmacology).*—Efficacy or otherwise of live vibrio cholera vaccines is demonstrated in a passive immunisation test in the infant rabbit against homologous and heterologous challenge. Adult rabbits were immunised with different types of live vibrio vaccines and the antisera obtained from these animals were used for passive immunization of a group of infant rabbits that were subsequently challenged intra-intestinally with live vibrios. The results were subjected to statistical analysis. In infected rabbits treated with antisera, three distinct responses were recognised. Some treated animals did not live longer than the untreated ones, some died but their death was delayed, and some survived the challenge. The potency of a vaccine was calculated according to two given formulae depending upon whether the animals survived or not. When the 95 per cent confidence intervals for the mean survival time between two groups of animals do not overlap, their mean survival times are taken as significantly different. Monovalent live vibrio vaccines of *V. cholerae* Inaba, *V. cholerae* Ogawa, *V. eltor* Inaba, *V. eltor* Ogawa and NAG strains demonstrated good antigenic value against homologous challenge but not against the heterologous challenge, except in the case of the vaccine containing *V. cholerae* Inaba 569B which showed significant protection against homologous and heterologous challenge with *V. cholerae* inaba, *V. cholerae* Ogawa, *V. eltor* Inaba, *V. eltor* Ogawa and NAG. The method is recommended to be studied for assay of cholera vaccines.

(ii) *Attenuation of V. cholerae with a view to produce live Cholera Vaccine (Departments of Pharmacology and Bacteriology).*—On repeated subculture in MacConkey's broth, it was observed that *V. cholerae* 569B (Inaba) strain lost its virulence. After 30 passages through MacConkey's broth, the LD₅₀ in mice of the passaged strain rose from 1000–2000 organisms to 10⁹ organisms. This strain also lost its cholera-genic property in the infant rabbit.

(iii) *Laboratory assay of field-tested Cholera Vaccines by infant rabbit method (Department of Pharmacology).*—A quantitative assay of vaccines used in the field in Dacca in 1963 and in Calcutta 1965 has been carried out as recommended in the bioassay technique given above. The laboratory assay values were compared with the relative effectiveness of the vaccines in the field trial.

The five cholera vaccines, one from Dacca (1963 field-trial) and four from Calcutta (1965 field-trial) that have been studied in the field were tested for potency by the infant rabbit protection test. The results of the rabbit tests and those in field trials were found to be comparable.

2. (i) *Studies on cholera toxin (Department of Pharmacology).*—Suitable media for production of cholera toxin. The preparation of a potent cholera-genic toxin in relatively pure form, requires that the organism be cultivated in a liquid medium. This is beset with difficulties to find out suitable media

which will give high yield of toxin. Several factors were considered and put to the test, viz., temperature of incubation, oxygen supply, time of incubation, rate of shaking during cultivation etc.

The following media were tried for preparation of the toxin:—

(i) Cohen and Wheeler's medium (modified by Bundeally and Rao) where several modifications were introduced.

(ii) Finkelstein and Lankford's medium.

The above media were modified by using (a) acid hydrolysed casein, (b) pressure hydrolysed casein.

Results

The media of Cohen and Wheeler (or its modification) in which *V. cholerae* were grown, were centrifuged. The supernates and the sonicated residual cells of these media did not produce choleraemic signs and symptoms in the infant rabbits.

(b) The modified medium of Finkelstein and Lankford (acid hydrolysed casein) in which *V. cholerae* was grown, was centrifuged. The supernate and the sonicated residual cells of this medium produced in the infant rabbits typical signs and symptoms of cholera.

(c) The modified medium of Finkelstein and Lankford (pressure hydrolysed casein) in which *V. cholerae* was grown, was centrifuged. The supernate did not produce any diarrhoeal symptoms in the infant rabbits. The sonicated residual cells produced only slight diarrhoea and some fluid accumulation in the large intestine.

(ii) *Intra-intestinal route of administration of ultra-sonic toxin.*—Intra-intestinal route of administration of ultra-sonic toxin in the infant rabbit was tried. Much lower dose of the toxin was required for producing diarrhoea in these animals as compared to oral feeding of the toxin. Only 1 ml/100g. of body wt. was found effective in producing profuse diarrhoea within 8 hours of administration as compared to 5 ml/100 g. of body weight when given orally.

(iii) *Purification of choleraemic fraction by chromatography.*—Sonicate toxin was prepared in distilled water and lyophilized. This material was used for the purification. The toxin was dissolved in very small quantity of saline and passed through G-200 Saphadex column. The column was eluted with normal saline and fractions of 5 ml. each were collected and spectrophotometric readings were taken. The fractions collected under different peaks were pooled, dialysed, lyophilized and reconstituted to suitable volumes and tested in animals. The fractions collected under 80 ml. to 190 ml. showed choleraemic actively in infant rabbits. The fraction collected under first 75-80 ml. showed antigenic activity as shown by Ochterlony's double diffusion technique.

(iv) *Absorption of toxin into blood from separated jejunal segment.*—One of the major unsolved problems in cholera research is whether the toxic agents primarily responsible for the diarrhoea are confined to the intestinal tract, or are absorbed into the blood. To solve this problem an attempt was made to prepare intestinal fistulae with the blood supply intact, in infant rabbits. A definite answer might be found to the question whether one of the important routes for the distribution of toxin was the blood. Vibrios, or their lysate were introduced into the separated segment with one end blind and the other opening to the exterior as a fistula. From thirty successful experiments it could be said that in infant rabbits a toxin of major importance was absorbed into the blood from *V. cholerae* in a separated segment of jejunum, and caused characteristic signs of cholera in the whole animal.

(b) Diphtheria Project

Aims and objects.—Haffkine Institute is interested in investigating epidemiological and public health problems as well as in the preparation of diagnostic reagents and prophylactic agents. The problems selected for investigation under the diphtheria project cover all these aspects. Some problems, such as the study of the role of phage in converting non-toxinogenic strains into toxinogenic strains, are of fundamental importance as well as of practical interest. It might help in the study of epidemiology of diphtheria because of the conversion by phage of non-virulent into virulent and toxinogenic strains. The development of phage typing of diphtheria strains as a result of this study would be of the interest in classification of diphtheroids. The study of the phage-host relationship itself is of fundamental interest in Bacteriology. The study may also result in development of methods for quick identification of virulent strains from throat swabs by using proper fluorescent tagged antisera. The host-phage relationship may also help in better study of toxin production by *C. diphtheriae*.

ACHIEVEMENTS SO FAR

1. *Role of phage in induction of toxin.*—Most workers in this field are of the opinion that the ability of phage to convert a non-toxinogenic strain into a toxinogenic strain is the inherent and inseparable property of the phage itself and is not due to transformation or transduction. However, the work carried out by Rajadhyaksha and Rao (J. Gen. Microbiol. 1965, 40, 421) has shown that the code for toxin production is provided by a toxinogenic factor termed T⁺ which could be separated from the organism or the phage, the phage acting merely as a transducing agent. It was possible to remove the toxinogenic factor from the phage and convert it into a non-toxinogenic phage by treatment with about 0.5 ug. of acriflavine *in vitro*. As far as is known, this is the first time that acriflavine is used for removing a transducible episome from a phage *in vitro*. It was also possible to remove from the organism, phage alone leaving the T⁺ factor or remove both by using different concentrations of acriflavine.

Another interesting aspect of the study of phage-host relationship was that the high toxin yielding strain PW8 contained the T⁺ factor and no phage. So far it was believed that this strain contained a defective non-inducible phage.

When the PW8 strain was infected with P phage the lysogenized strain produced less toxin (Rajadhyaksha and Rao, J. Gen. Microbiol., 1965 40, 421).

2. *Role of iron in the control of toxin production.*—It is well known that for optimum production of diphtheria toxin by the PW8 strain, iron in the medium should be adjusted to a narrow margin. Many theories have been put forward such as (i) it combines with intracellular toxin preventing its excretion into the medium; (ii) it affects phage multiplication; (iii) it inhibits the code for toxin production either by combining with DNA or RNA or by inhibiting the polymerase; (iv) it acts as a corepressor; (v) it is the protein moiety of cytochrome and is excreted at low iron levels. None of these theories have been substantiated by experimental results and the mechanism of how iron affects toxin production was not known.

Our preliminary results using PW8LR strain showed that the concentration of iron required for optimum toxin production is the same whether iron is added in Ferrous or Ferric state. Further, it was found that the effect of excess iron can be nullified by adding small amounts of either EDTA or acriflavine to the medium. Kinetic experiments revealed that change of iron concentration in the medium from optimum to excess or *vice versa* after 24 hour of growth, took at least another 24 hours for its effects to be reflected in change in toxin production. These experiments were suggestive of the fact that iron may act extracellularly rather than intracellularly.

The isolation of PW8 Cys—strain, which grows in presence of cystine, glutamic acid, nicotinic acid and salts, provided an opportunity to test the effect of iron on toxin production. Results showed unequivocally that iron acts by its ability to complex with cystine which is an essential nutrient for the RW8 Cys. strain. Experiments using fixed levels of cystine and varying levels of iron showed that the amount of iron required to be added for getting optimum toxin depended on the level of cystine in the medium. Similarly, even at inhibiting levels of iron, toxin can be produced with largest amounts of cystine in the medium. It was also found that largest amounts of toxin is produced by the PW8 Cys—strain in the absence of added iron if cystine concentration is 0.01g per cent. Results are summarised in Tables I and II. It is to be assumed that cystine-iron complex is not permeable into the cell unlike cystine. Iron in the medium, therefore, acts by regulating the amount of cystine available to the organism for growth and toxin production.

TABLE I

Level of cystine (g %)			Opt. con. of iron mg/litre	Toxin (Lf units)
0.01	...	...	<i>Nil</i>	50
0.015	...	...	0.015	45
0.03	...	...	0.025	45
0.08	...	...	0.07	15

TABLE II

Level of Fe (mg/litre.)				Opt. con. of. cystine (g%)	Toxin (Lf/ml)
0.0	...	...	...	0.01	50
0.125	...	...	...	0.025	36
0.3	...	...	...	0.04	40

PROBLEMS UNDER INVESTIGATION
IMMUNOLOGY DEPARTMENT

1. *The conversion of non-toxinogenic diphtheria into toxinogenic strain the mediation of phage.*—It has been shown in our laboratory that phage the role of transducing the toxinogenic factor T⁺ while converting a genic strain into toxinogenic strain. The rate of transduction is over 90 per which is unusually high. It was, therefore, felt that T⁺ factor may have mechanism for its transfer by cell contact without the help of phage. Such transfer has been observed in the transfer of sex factor and colicinogenic factors in *E. coli*. Preliminary results showed that such a transfer was possible, Experiments are being planned to test the effect of actual cell contact. Efforts will be made to see whether DNA from a toxinogenic strain will transform a nontoxinogenic strain into a toxinogenic strain.

2. *Role of iron in controlling toxin production by C. diphtheriae.*—It has now been fairly well established that iron in the medium acts by with cystine as already mentioned before. However, it is necessary to see whether there is a stoichiometric relationship between cystine content and its inhibition by iron. Our previous experiments showed that cystine is permeable into the cell but iron cystine is not. This has to be verified by using isotopes of cystine and iron.

3. *Isolation of diphtheria strains useful in production and for*
Three useful strains have been isolated so far. Starin PW8LR is able to on protein-free medium based on acid hydrolysed casein under shaken tions. It is able to produce 60 Lf/ml. toxin under shaken conditions 96 hours with fractional addition of Maltose. The strain used in production at the Institute produces about 60 to 80 Lf/ml. after 7 days stationary cultures.

Maltose is used by all laboratories engaged in diphtheria toxin as it is the best source of energy for the organism. However, it has some advantages. It has to be deferrated before use. It has to be sterilized and added to the medium just before inoculation. The quality of maltose known to vary from batch to batch. Another important reason for us substitute Maltose is because Maltose has to be imported with our foreign exchange. Efforts were, therefore, made to obtain a strain capable utilizing sucrose. Among the strains in our possession PW8LR was capable

utilising sucrose very slowly. A variant was isolated from PW8LR which was labelled PW8LRS. It could give about 20 Lf/ml of toxin in 0.5 per cent sucrose casein hydrolysate medium under shaken culture condition in 4 days. By adjustment of various constituents of the medium, it is now able to produce 40 to 45 Lf/ml. If its performance can be improved still further to above 60, it could be taken up for routine production of toxin.

A strain labelled PW8 Cys—was isolated. It is capable of growing and producing toxin in a simple medium containing cystine, glutamic acid, nicotinic acid and salts. It was able to produce under shaken conditions about 20 to 25 Lf/ml of toxin. This strain was very useful for the elucidation of the role of iron in the control of toxin production of by *C. diphtheriae*.

4. *Measurement of the number of antigens induced by T⁺ factor.*—The culture filtrates of PW8T[—] and PW8T⁺ were analysed in Ouchterlony gel diffusion plates using horse anti-diphtheria serum. PW8T[—] strain showed 8 lines and PW8T⁺ showed 11 lines. Because of the interference of the antigens produced by the organism the identification of antigens produced by the T⁺ factor is difficult. To avoid this difficulty absorption technique was used. The horse antisera were absorbed with the required quantity of concentrated culture filtrate from PW8T[—] strain till it showed no reaction in gel diffusion with the concentrated culture filtrate of PW8T[—]. When these absorbed sera were used, PW8T⁺ culture filtrate showed one prominent line due to toxin and two minor lines due to two antigens which are as yet unidentified. It will be worthwhile investigating what these additional antigens are.

5. *Use of adjuvants and hormones to study the stimulation of antibody production to diphtheria toxoid in rabbits.*—It is known that diphtheria toxoid is a poor antigen and adjuvant is usually used in human or horse immunization. A number of adjuvants such as aluminium phosphate, calcium phosphate, alginate, Freund's incomplete adjuvant, Freund's complete adjuvant, dianabol (Ciba) and millicorten (Ciba) were used in groups of 10 rabbits. Best results were obtained with dianabol administered orally and with complete Freund's adjuvant. Further work is progressing.

6. *Study of cellular constituents of C. diphtheriae (Biochemistry Department).*—Study of cellular constituents may throw light on the metabolism of *C. diphtheriae*. Five batches of cells of PW8 strain grown for 7 days in different media were analysed for total nitrogen, protein, RNA, DNA, total sugars, lipid phosphorus and iron. Four batches were grown on Holt's medium and one on the papain digest of meat medium. There was wide variation in the results probably because of lysis in a considerable number of cells during the seven-day period.

7. *Phage typing of C. diphtheriae. (Clinical Pathology Department).*—Work on this problem was started only in October 1966. Using Hoyle's tellurite medium, a few strains are isolated with their clinical history. An attempt is being made to standardize Groman's method for determining the toxicity of these strains. The work is just in progress.

(c) Enterovirus Project

(1) PROBLEM OF GASTROENTERITIS : (VIRUS DEPARTMENT)

Aims and Objects.—Aetiology of gastroenteritis, particularly in infants and children in a large number of cases, is obscure. Suggestive evidence for relationship of certain Echo virus types to undifferentiated summer diarrhoea of childhood has been put forward by some workers. It is also postulated the clinical syndrome of undifferentiated acute gastroenteritis in children be as a result of a symbiotic action of an enterovirus and enterocoli.

Few workers in India have tried to investigate the problem of viral etiology of gastroenteritis but no systematic investigation has been carried out so far.

The aim of the present project is to investigate the problem of acute gastro enteritis in children from viral etiology.

Selection of cases.—Cases were selected by a paediatrician. All cases dysentery (Bacterial or protozoal) were excluded from the present study. After this, preliminary screening material for virological studies was collected from the undifferentiated diarrhoea cases.

Collection of materials.—Starting from September 1965 upto September 1966 materials from 206 cases of acute gastroenteritis were collected.

In addition to the materials from cases of gastroenteritis, samples from 170 control children were also collected. Controls were selected as the basis matching age groups socio-common status, geographical distribution and period under study.

Methods.—For virus isolation, stool suspension or rectal swab suspension is inoculated in Rhesus Monkey Tissue Culture and infant mice. All are carried for two blind passages before being labelled as negative. For identification, a preliminary screening against pooled and individual antisera is carried out. Those failing to be neutralised with polio are put up against grups of Coxsackie and Echo antisera. Further, isolates are studied for haemagglutination and HAI activity.

Serological studies.—To determine the specific role of a viral isolate the clinical syndrome, paired samples of sera are collected at an interval of two to three weeks and these are to be tested against the viral isolates for rise specific antibodies.

Results.—Results of studies carried out on cases of gastroenteritis as well controls are summarized in the following table:—

Table showing results of virus studies

		Total No.	No. + ve for entero- virus.	+ve in TC alone	+ve in infant mice alone	+ve both in TC and and mice
Gastroenteritis	..	206	199 (97%)	118* (58%)	13 (6.5%)	68 (35.5%)
Controls	..	170	34 (20%)	14 (41%)	18 (53%)	2 (6%)

*% of positive.

Identification of the virus isolates is still in progress. Details of the identification carried out so far are as follows :—

Polio type 1	.. 1	Cox A9	.. 4
Polio type 3	11	B1	.. 1
Cox A2	.. 2	B2	.. 1
A5	.. 2	B3	.. 8
A6	.. 4	B4	.. 4
A8	.. 8	B5	.. 1
A10	.. 4	Echo type 6	.. 2
A23	.. 1	Echo type 7	.. 69

Identification of 91 strains isolated in tissue culture and 52 strains isolated in mice is yet to be carried out.

Haemagglutination studies.—186 strains isolated in tissue culture have been tested for HA activity using Human "O" group cells. 127 strains have been found to possess HA activity while 59 strains did not show any HA. Titres of HA were found to be in the range of 1:8 to 1:128.

Observations.—From the studies carried out so far, following observations are drawn :—

(1) Cases of gastroenteritis show a significant virus isolation rate (97 per cent) when compared to that of the controls (20 per cent).

(2) The number of individuals showing presence of virus only in mice is also significantly different in two groups. 13 cases out of 199 virologically positive in the gastroenteritis group (6.5 per cent) and 18 out of 34 positive controls (53 per cent).

(3) It is interesting to note the large preponderance of single serotype (Echo 7) among the cases of gastroenteritis.

Though the study is still incomplete, it appears at this stage that Echo 7 type seems to have some etiological significance in the clinical entity of acute gastroenteritis in children. It would be of interest to note that Echo type 7 has so far not been attributed to any clinical syndrome in human beings.

Symbiotic relationship between an enterovirus and enterocoli (C.P.).—To explore the possibility of any symbiotic relationship between an enterovirus and enterocoli in producing the clinical syndrome of gastroenteritis, bacteriological studies were also undertaken on the same samples.

Results obtained in the present study have failed to indicate any such relationship. Bacteriological studies have since been discontinued.

Gastroenteritis in Calcutta (VIRUS DEPARTMENT)

This study was undertaken in collaboration with the Johns Hopkins Medical Centre in Calcutta.

Cases of gastroenteritis in adults, which clinically appear to present a of cholera but with negative bacteriological findings, have been noted workers in Calcutta. It was decided to investigate these cases from view-point.

One batch of 20 fecal samples was received at H.I. and from the undertaken on these 20 samples, 16 have been found to be in tissue culture. Seven isolates have been found to be showing tion with human "O" group cells. Identification of these isolates gress. Isolation of an enterovirus from 80 per cent of cases which have bacteriologically negative is a significant finding. To assess the importance these findings, fecal samples from cases of cholera which have been bacteriologically positive for *Vibrio cholerae* were obtained from 39 samples were recieved of which 9 have proved to be virologically positive. Further studies are in progress.

(2) ASEPTIC MENINGITIS (VIRUS DEPARTMENT)

Aims and objects.—The present study was undertaken to explore the bility of viral etiology of the clinical syndrome 'Aseptic Meningitis' in

C.S.F., Throat Swabs, Rectal Swabs and blood samples were collected from 160 cases of suspected aseptic meningitis. A total of 336 samples have been collected so far.

Using monkey kidney tissue culture, infant mice and adult mice as host media, materials collected are being inoculated for virus isolation. Results obtained so far are summarised below :—

Aseptic Meningitis

Type of the specimens	No. of samples collected	No. of samples Tested			No. of positive samples			Identified	Awaiting results
		Tissue Cultures	Infant mice	Adult mice	Tissue Cultures	Infant mice	Adult mice		
Cerebro spinal fluids.	225	162	75	95	24	4	0	4 Cox B ₁ -1 Echo 4-1 Echo 19-1 Echo 21-1	24
Rectal Swabs ..	164	108	64	75	20	2	0	5 Echo 19-4 Echo 21-1	17
Throat Swabs	101	96	56	66	16	2	0	4 Cox B ₁ -1 Echo 1-1 Echo 18-1 Echo 19-1	14
Total ..	490	366	195	236	60	8	0	13 Cox B ₁ -1 Cox B ₂ -1 Echo 1-1 Echo 4-1 Echo 18-1	55

Identification of viral isolates.—29 strains isolated in tissue culture have been put up against polio antisera and all have proved to be non-polioviruses.

Further work is in progress.

Observations.—It is premature to draw any firm conclusions from the results obtained so far. Isolation of 15 viruses from the C. S. F. is a fairly contributory finding.

(3) STUDIES ON ENTEROVIRUS TRANSMISSION BY SEWAGE.— (Polio Unit)

Sewage samples were collected as described earlier, to assess the possible role of Sewage pollution in enterovirus transmission.

36 more samples were collected and tested for the presence of Enteroviruses. The results are as follows:—

Total No. of samples collected	...	...	...	100
No. of samples tested in TC + Mice	...	...	...	100
No. of + ve in TC	...	...	...	83
No. of + ve in infant Mice	...	...	...	17
No. of samples + ve in both TC + Mice	...	...	...	13
No. of samples + ve only in Mice	...	...	...	4
No. of samples identified	...	...	...	54
Polio-I	...	...	...	10
Polio-II	...	...	...	1
Polio-III	...	...	...	9
Cox B ₁	...	...	...	3
Cox B ₂	...	...	...	1
Cox B ₃	...	...	...	3
Cox B ₄	...	...	...	20
Echo 7	...	...	...	5
Echo 9	...	...	...	6
Echo 10	...	...	...	2
Echo 18	...	...	...	3

Of the virus isolates from 54 samples identified, 7 samples showed the presence of 2 or more viruses. Thus making the total viruses isolations to 63.

(d) Plague Project

(1) *Rats and Fleas (Entomology Department).*—Plague studies were carried out in the Marathwada region of Maharashtra State following places, viz. Naldurg, Osmanabad, Murud, Ambejogai, Bhir Gevrai, Shahagad, Pachod, Daulatabad, Sarai, Sillod and Ajanta.

The different species of rats collected and fleas recovered from these rats indicated in Table I.

TABLE I

Trapping—

Trapping days	...	...	...	...	67
Trapping No. of places	...	...	...	...	10
Total bait material used to collect rats in traps	...	...	...	...	45 kgs
Total rats collected in traps	...	...	...	...	2,324

Different Sp. of rats trapped.

Kind of rats				Male	Female	Total
<i>Rattus rattus</i>	..	..	..	992	1,264	2,256
<i>B. bengalensis</i>	..	..	..	1	2	3
<i>L. hooduga</i>	..	..	..	2	2	4
<i>T. indica</i>	..	..	..	5	2	7
<i>G. ellioti</i>	..	..	..	2	1	3
<i>B. blendfordi</i>	..	..	..	2	2	4
<i>M. musculus</i>	..	..	..	25	22	47

Total fleas collected—8,118.

Different species of fleas collected.—

				Male	Female	Total
<i>X. astia</i>	..	..	..	131	166	297
<i>X. cheopis</i>	..	..	..	3,824	3,992	7,816
<i>E. gallinacea</i>	..	..	..	1	3	4
<i>Hosopsyllus faciatius</i>	..	..	..		1	1

*Rat Control—**Baiting—*

A. Zinc phosphide	...	...	...	in Bait boxes.
Baiting days	...	...	...	72
Baiting places	...	...	...	19
Total bait used	...	...	...	65·425 kg.
Total Zinc phosphide used in these baits	...	...	...	1·025 kg.
Total rats collected as dead early in the morning.	...	...	...	489
Cost of grain at 75 paise	...	...	...	Rs. 50 approx.
Per kg. cost of Zinc	...	...	...	Rs. 15 approx.

B. *Rodafarin.*—

Period	...	...	...	19 days.
No. of places	...	...	...	2
Baits used	...	...	...	19 kg.
Ready-made bait cost	...	...	...	Rs. 25.
Total dead rats collected	...	...	...	23

The two common methods of rat control i.e. trapping, baiting were tried. Two rodenticides, viz. Zinc phosphide, Rodafarin were tried in the field to see how they could be used under rural conditions.

Baiting campaigns were taken up after arousing enthusiasm amongst local people in some of the villages. These were carried out by villagers themselves under guidance.

(2) *Rat susceptibility (Bacteriology Department).*—The rats from different places were challenged to a standard dose (5,000 organisms) of *P. pestis* in order to assess their susceptibility status of plague. The results are given below in Table II.

TABLE II

Results of susceptibility of Rats (Rattus rattus) to P. pestis (195/P)

	Date of inoculation	Number of rats inoculated			Number of rats died within 15 days			Number of rats found positive			Per cent susceptible	Per cent Resistant
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Total	Total
Osmanabad	.. 9th January 1967.	42 *2	42	84 *2	15	17	32	11	14	25	30.5	69.5
Murud	.. 20th January 1967.	32	32	64	12	15	27	9	13	22	34.4	65.6
Pali	.. 7th February 1967.	10	10 *1	20 *1	3	3	6	2	3	5	26.3	73.7
Ambejogai	.. 7th February 1967.	30 *2	30 *2	60 *4	9	13	22	8	9	17	30.3	69.7
Gevrai	.. 6th March 1967.	30 *1	30	60 *1	18	16	34	11	11	22	37.3	62.7
Shahagad	.. 6th March 1967.	15 *1	15 *1	30 *2	6	6	12	5	4	9	32.1	67.9

From the results obtained it is noted that very few rats have been challenged endemic forest areas. In the next studies in the Western Maharashtra these studies need intensification.

The susceptibility tests on rats from Marathwada area were continued using challenge of approx. 5,000 organisms as the challenge dose. While of 22-25 grams of N. I. H. strains were used as control for the challenge a dose of 10-20 organisms killed 80-100 per cent of the mice used.

The results obtained are presented in the accompanying table.

It will be observed that rats from all the places show a high resistance of 70 per cent. The blood samples of some of these rats have been collected and will be tested by haemagglutination test for presence of antibodies.

The antiplague serum in rabbit has been prepared and also the fraction I antigen is prepared. The antiplague serum was tested by Macrohaemagglutination test which gave a titre of 1/5,000 while normal rabbit serum showed a titre of less than 1/10. The specificity of the test was thus confirmed.

Plague Vaccine (Analytical and Control Department).—The work on of a standard vaccine for assay of plague vaccines was carried out in two parts. These studies are to be further continued.

The first part of the problem, viz. testing of 5 random batches of plague and picking up a batch which gives a mean protective dose (ED 50) as a batch for use as a standard vaccine is over. The results of the assays of the 5 batches are given in the table III below :—

TABLE III

No. of vaccine	E.D. 50 of Vaccine in ml Expt. Nos.					
	1	2	3	4	5	6
1A	0.0055	0.0092	0.013			
3A	.. 0.008	0.0082	..	0.01	..	..
48-10	.. 0.008	0.018	0.022	..	..	..
48-15	..		..	0.011	0.0086	0.016
48-25	..		..		0.011	0.018
140	..		0.004	0.007	0.0021	0.008

It is seen from the results given in the table that out of 5 batches, 4 batches give fairly close ED 50 values and batch 48-10 gives a slightly higher ED 50 value showing that the batch may be slightly less protective than the other four batches. Therefore any one of these four batches can be used as a standard vaccine. This also incidentally shows that the production of plague vaccine is well standardised and batches prepared at different times are fairly uniform in quality.

The work on the 2nd part of the project is in progress.

(4) *Biochemical Studies (Nutrition and Biochemistry Department).*—The biochemical studies on the growth factors in the casein hydrolysate media continue. The effect of Tryptophane and phenyl alanine needs further studies.

To sum up, the work pertaining to various aspects of plague epidemiology was carried out under the plague project. It is suggested that more work should be done in the other parts of Maharashtra region, in the deep forest plague endemic areas, on the collection of rats, their population, their susceptibility status to plague, and locating hidden infections and rat control methods and rodenticides need to be specifically tried.

B. OTHER DEPARTMENTAL RESEARCH

(1) Department of Bacteriology

1. *Acetone-killed and Acetone-dried Typhoid Vaccine.*—The work on preparation of acetone-killed and dried vaccine was continued. The method followed initially was the same as that described by the WRAIRL with the modification that use of carbontetrachloride as a suspending agent was omitted owing to its toxic nature and large requirements. The final suspension in ten times the final strength was prepared in acetone and rapidly dispensed into vials when the suspension was kept rapidly stirred with a magnetic mixer. The process followed for drying was the same as described, *i.e.* evaporating the acetone at 37°C by leaving the vials in an incubator overnight and effecting the final drying under vacuum. When this procedure was followed it was observed that the final product obtained could not be re-suspended easily in normal saline. To overcome this difficulty, addition of several substances such as Tween 80 and wetting agents like sodium taurocholate were tried but there was no improvement in solubility. Secondly, after final washing with acetone, acetone was completely removed under vacuum and then this product was suspended in 1 per cent mannite and was lyophilized. This gave the product of desired solubility. However, this procedure involves the step of lyophilization of the product which is labourious and also requires special freeze-drying equipment for large-scale production. This, in fact, offsets the advantage obtained by acetone process.

It was observed during the step of dissolving the product in mannite that the product, after quick-drying, from acetone solution, suspends quickly in solution of mannite. This gave a clue for further trials. In further experiments the suspension of the organism in acetone was distributed in vials which were quickly dried directly under vacuum without keeping the dispensed suspension overnight. This improved the solubility of the product considerably. At about the same time, Dr. Lowenthal of WRAIRL visited on behalf of the W.H.O. After personal discussions and trials of drying the lots, it was observed that high humidity in Bombay interfered with the drying procedure as described by WRAIRL giving the final product which could not be suspended. Too rapid drying under vacuum was also not desirable. Taking these factors into account, several lots of vaccine have been prepared which suspend uniformly within a minute. The product also is tested for sterility, toxicity and mouse protection test and gives satisfactory results.

Further work on adjusting the concentration of organisms by methods other than opacity is in progress.

2. *Immunization with a combined T.A.B. Cholera Vaccine.*—Combined prophylactics such as D.T.P. or T.A.B., Cholera Vaccine are of considerable help in public health immunization programme in our country and a question is always raised, especially during epidemics, if T.A.B. and Cholera Vaccine can be mixed and injected at one site. Secondly, a combined T.A.B. Cholera Vaccine is described in B.P. 1963. It is observed in the case of use of combined prophylactics that some interference in immune response to one of the antigens may be produced if the proportion of individual components is not properly balanced. Therefore, with a view to have some idea about the immunizing capacity of such a preparation, the following work was undertaken :—

Three groups of rabbits, each consisting of three rabbits, were immunized with classical (1) Cholera Vaccine, (2) T.A.B. Vaccine and mixed T.A.B. Cholera Vaccine respectively. The vaccines were injected in two doses of 0.5 and 1 ml. at intervals of one week. A sample of blood was collected from each of the rabbits before injecting the vaccine. After immunization, blood samples are collected at an interval of one week, two weeks and four weeks and 2 months and 3 months. The sera are separated and agglutination tests are put up using the following antigens :—

(a) *S. typhi* O, (b) *S. typhi* H, (c) *S. typhi* Vi, (d) *S. paratyphi* A, (e) *S. paratyphi* B, (f) *V. cholera* Inaba, (g) *V. cholera* Ogawa.

In the first group of rabbits, immunized with cholera alone, two rabbits died during collection of the blood and after 1st and 2nd week, and results for other rabbits are available for sera up to 4 weeks after immunization. The geometric mean titres of three rabbits immunized with T.A.B. Vaccine and mixed T.A.B. Cholera Vaccine are given below :—

Antigen		Pre-immunization week	T. A. B. Vaccine			Mixed T. A. B.-Cholera			
			Geometric mean titre			Geometric mean titre			
			1 week	2 weeks	4 weeks	Week	1 week	2 weeks	4 weeks
<i>S. typhi</i> O	..	1/32	1/27	1/254	1/253	1/12	1/254	1/160	1/160
<i>S. typhi</i> H	..	1/10	1/800	1/403	1/201	1/10	1/1016	1/507	1/507
<i>S. typhi</i> Vi	..	1/10	1/80	1/100	1/100	1/10	1/201	1/100	1/160
<i>S. para-typhi</i> A	..	1/10	1/507	1/253	1/320	1/10	1/806	1/507	1/320
<i>S. para-typhi</i> B	..	1/20	1/1016	1/403	1/254	1/10	1/639	1/507	1/403
<i>Cholera</i> Inaba	..	1/10	1/10	1/10	1/10	1/10	1/403	1/320	1/253
<i>Cholera</i> Ogawa	..	1/10	1/10	1/10	1/10	1/20	1/639	1/253	1/320

The results show that as far as agglutinin production is concerned, the addition of cholera vaccine to the T.A.B. vaccine does not make any difference.

The geometric mean titres of rabbits immunized with T.A.B. vaccine alone are not significantly different from those obtained when combined prophylactic is used. The results as far as cholera vaccine is concerned show the same trend. However, these tests will have to be repeated with larger number of rabbits to arrive at any definite conclusion.

3. *Preparation and standardisation of typhoid vaccine.*—The typhoid vaccine produced at the Institute is prepared from two standard strains of *S. typhi* and one each of *S. paratyphi* A and B. The killing agent is formalin and preservative used is phenyl mercuric nitrate. The vaccine is also standardised on the basis of total count as determined by opacity methods.

It is a common belief in some quarters that the strains isolated locally may offer better protection if they are used for preparation of vaccine than the standard strains selected in some foreign countries. As there is no reliable laboratory method for testing the potency of typhoid vaccine, the only other alternative is to test its efficacy in strictly controlled field trials. These trials, however, are too expensive and require elaborate technical skill and are not always possible. Thus the need for evolving a suitable laboratory method which will reflect the efficacy of the vaccines in human beings is self-evident.

Secondly, the controlled field trials in Yugoslavia and New Guinea have shown that none of the present laboratory methods is suitable for standardisation of typhoid vaccines. Therefore, a newer method other than those in current use is an urgent necessity. Thus, with a view to study these two points, the following investigations were undertaken. The plan of work adopted was to prepare two types of vaccines (1) Heat killed and phenol preserved and (2) Formalin killed and p.m.n. preserved using standard *S. typhi* strain Ty2 and two locally isolated strains and test their potency by (a) agglutination tests (b) active mouse protection test (c) by a new method, active or passive rabbit intracutaneous test.

The vaccines were prepared by the methods mentioned above using standard strain Ty2 and two locally isolated and properly tested *S. typhi* strains.

The vaccines passed satisfactorily the sterility test and toxicity tests in guinea-pigs and mice.

For determination of potency, all the tests mentioned above were used. The results obtained are given below :—

(1) *Agglutination production in rabbits.*—Normal healthy rabbits were immunized by intravenous injection of different vaccines using same doses as used in W.H. O. trials in Yugoslavia and other places. The agglutination tests were carried out against standard *S. typhi* suspensions O, H, and V_i.

The agglutination titres produced in rabbits against the three antigens were not of any help in differentiating the vaccines. The O antibody titre was round about 5120 for phenolized vaccines produced from different strains. Though minor differences were observed in titres, they were not of any statistical significance. The formalized vaccines showed a titre of about 1/2560.

The "H" antibody titre was about 10240 in cases of formalized vaccines, while the titre was about 2560 in the case of phenolized vaccines. The Vi antibody titre for both the groups of strains was about 640 for all the vaccines. Thus the agglutinin production was not of any help in distinguishing between two vaccines.

(2) *Active mouse protection tests.*—The active mouse protection test gave an ED_{50} of 0.004 or less for formalized and phenolized vaccines prepared with the standard strain. The phenolized vaccines showed a lower ED_{50} than formalized. However, the significance of these results will be assessed after few more tests. In contrast to this, the local strain vaccines showed an ED_{50} of 0.006 or more indicating that this method may be useful for distinguishing poor vaccines from good ones. However, statistical analysis will only provide the final answer after some more repeat tests.

(3) The vaccines were tested by intracutaneous challenge in rabbits. This method has been now standardized. The graded doses of cultures mixed with mucin show graded lesions in the skin starting from a red flush and slight swelling passing through lesions of increased diameter, erythema and induration and finally resulting in necrotic lesions. The immunized rabbits show no lesions with lower doses of the culture and even higher doses show milder type of reactions and rarely necrosis, when a dose as high as 80 MRDS is used.

In the present series of experiments, the rabbits were immunized with different doses of vaccines given intravenously and challenged with increasing doses of the mucinized culture dilution. The results show that vaccines prepared from standard strains showed a neutralization of 20 MRDS, while definitely positive reaction is obtained with 40 and 80 MRDS. There was no marked difference in results obtained with either formalized or phenolized vaccines prepared from standard strains. On the contrary, the vaccines prepared from local strains show a neutralization of only 10 MRDS or less. Thus, it is possible to distinguish by this method between the poor and good vaccines. Thus, by selecting a proper challenge dose, it may be possible to grade the vaccines in respect of their potency.

(4) Passive neutralization test using intracutaneous test also gives similar results, though the level of neutralization obtained is lower.

(5) With a view to study the effect of route of inoculation of vaccines on the results of intracutaneous test, a preliminary experiment was carried out using 3 vaccines (i) acetone-killed and dried vaccine from Walter Reed Army Research Institute, (ii) Routine Formalized Vaccine, (iii) heat-killed and phenol-preserved vaccine. The strain used for preparation of vaccine was Ty2. The results showed that WRARI vaccine showed a neutralization of 40 MRDS as against 20 MRDS shown by routine and heat-killed vaccine. This again shows that the method is able to distinguish between vaccines. Further work on modification of this test, so as to reflect the potency of the vaccines in a better way, is in progress.

(2) Department of Blood Bank

1. *Bacterial contamination of banked blood.*—This problem has been up for further detailed study to trace the sources of contamination and evolve methods to avoid them in large-scale collection of blood in sessions. This includes the study of—

- (i) Sources of contamination and nature of contaminations.
- (ii) Isolation of organisms from different sources such as donor's place of work, etc.
- (iii) Typing of these organisms.
- (iv) Methods to be evolved for reducing this chance contamination.

From the above study, the isolation and typing of organisms is going on. So far, 291 samples of contaminated blood are studied from different collection of blood such as (i) Haffkine Institute, (ii) Jails, (iii) St. Hospital, (iv) Tata Blood Bank, (v) K. E. M. Hospital etc. centres.

It was found that the common contaminants are staphylococci, *E. coli*.

The following table shows the number of isolations:—

<i>Staph. albus</i>	...	...	...	...	54
<i>Staph. citreus</i>	...	...	...	...	15
<i>Staph. aureus</i>	...	...	...	...	45
Fungus	...	...	...	...	38
Spores (Aerial)	...	...	...	...	29
<i>E. coli</i>	...	...	...	...	15
Total					196

The antibiotic sensitivity test is to be carried out for these different organisms. Steps are being instituted to reduce these contaminations by improving the methods of collection and transport of blood.

2. *Preparation of anti-Rh serum.*—In order to prepare pure anti-D serum, professional Rh negative donors (cde/cde) were given weekly injections of O Rh+ve blood. Nineteen Rh negative donors consented to be immunised and of these 7 donors took a couple of injections each and did not turn up for further immunisation. Out of twelve donors that continued to take the injections, three have made sufficiently high titre anti-D. These have been bled every month about 300 ml. So far, we have about 2,000 ml. of pure D. As these donors all do not belong of AB group, some of the serum has been absorbed with the proper Rh negative cells. (The remaining nine donors are still receiving weekly injections of the Rh+ve cells.) So far, we have about 1,000 ml. of absorbed anti-D and 1,000 ml. of unabsorbed anti-D with us. The first batch of pure anti-D will be ready for supply in May 1967. Sufficient quantity of Anti-C+D is in stock to meet the demands.

So far, the method recommended for testing is the Albumin Replacement Technique. This method is long and requires the use of albumin. At present, a rapid method to do this test is being worked out and also for preparation of serum suitable for rapid slide test.

3. *Standardisation of Coomb's Reagents*.—The improvements in the methods of the production of anti-human globulin serum has been undertaken by using different fractions like gamma globulin and albumin as antigens in place of whole human serum. This will enable us to get anti-gamma and anti-non-gamma type of Coomb's reagent.

4. *Preparation and standardisation of other blood group reagents*.—
(i) Preparation and standardisation of Anti-M and Anti-N Sera (Rabbits)—

(a) *Preparation of Anti-M Serum*.—For this 9 rabbits were immunised out of which 5 rabbits were immunised by Mollison's Technic and 4 by Weiner's Technic. The rabbits have been given four courses of immunisation so far and the Anti-M titre after observation is 1:16. Another course of immunisation has been given to boost the titre.

(b) *Preparation of Anti-N Serum*.—For this 9 rabbits have been immunised by Mollison's technic, and 2 by Weiner's technic. So far, 4 courses of immunisation have been given and all rabbits have been given fifth course of immunisation by a modified technic as prescribed by Miss Elizabeth Naney of Ortho-Pharmaceuticals, New York, U. S. A.

(ii) *Preparation of Anti-A₁ (human)*.—For this a high titre anti-A serum was selected which was absorbed by A₂ packed cells, to remove A₂ agglutinins; so far 500 ml. of Anti-A₁ serum has been produced.

(iii) *Preparation of Anti-A₁ (Lectin)*.—*Dolichos biflorus* seeds have been used for this production. They are extracted with saline and the extract is processed further. 200 ml. of this lectin has been prepared so far.

(iv) *Preparation of Anti-H (Lectin)*.—*Ulex europeus* seeds have been used for this production. They are extracted with saline and the extract is processed further. 100 ml. of this lectin has been made so far.

(v) *Preparation of Anti-H (Fowl)*.—Two fowls have been injected with OH+ve bloods. So far 6 injections of 1 ml. each have been given intravenously through wing vein. The serum of the fowl will have to be absorbed with Bombay phenotype bloods to produce anti-H specific finished product.

(vi) *Blood Group Substance*.—The preparation of blood group specific substances from meconium by alcohol precipitation method has been undertaken. The meconium is being collected from N. Wadia Maternity Hospital, Bombay.

5. *Bovine Albumin*.—Bovine albumin prepared by Howe's ammon. sulphate method was not giving satisfactory results consistently. There was variation from batch to batch. Bovine albumin is now prepared by Cohn's alcohol precipitations method. The final product was freeze-dried and dissolved in buffer at pH 7.5 at 22 per cent strength and this was used as blood grouping reagent.

6. *Study of Genetical Characters in some endogamous group in Indian population.*—This study of blood groups and other genetical characters of certain endogamous groups in Indian population is in progress. The following characters are being studied. Not less than 200 subjects in each group are studied for each genetic character:—

- (i) Blood groups ABO, Rh, MNSs, Duffy and Kell system.
- (ii) Glucose six phosphate dehydrogenase tests.
- (iii) Phenyl thio Carbamide test.
- (iv) Secretor and non-secretor status.
- (v) Haemoglobin and Sickling.
- (vi) Haptoglobins.
- (vii) Colour blindness.

The study of three hundred bloods of three different communities from Gujrat have been examined, viz. Visa Oswal, Jains, Audich Brahims and Lad Vania for G-6-PD enzyme deficiency, haptoglobins and abnormal haemoglobins.

There is very low frequency of G-6-PD deficiency (1-2 per cent) in these communities.

Haptoglobins.—The study on about 200 serum samples from the three Gujarati groups mentioned above revealed a uniform finding. There was a low frequency (0.1) Hpl. gene. This finding is in agreement with other reports on Hpl frequency in Indians but sharply contrasted with that in Negroes who have a high Hpl frequency (0.5-0.7). Recent studies in other countries have shown to have some relationship with endemic malaria. Further studies will reveal how far this is true in our country.

Abnormal Haemoglobins.—No abnormal Haemoglobin with a different mobility other than that of Hb-A was encountered.

Blood Groups.—225 Syrian Christians have been studied. Among the ABO system O group is predominant while distribution of B group is little higher than that of A group.

All the above results will be analysed statistically when the required number of samples in each group will be completed. They will be compared with general pattern of distribution of various characters in the world.

7. *Tissue Banking.—Bone Grafts.*—The ribs removed at thoracoplasty operations were obtained from R. P. T. B. Hospital. They were preserved in antibiotic solution. They were further processed and cut into match stick size and then freeze-dried for 7 days. The moisture content was determined and was found to be 2.5 per cent which is quite satisfactory. The sterility tests

were also carried out on the final samples (grafts). The grafts, which pass all these tests were supplied to the orthopaedic surgeons for clinical trials. In 6 cases these grafts have been used as supporting grafts in bone so far. In one case, a gap of 4 inches in the bone has been filled up with these grafts. So far no infection or any immunological reaction has been observed in any of these cases. The post-operative X-ray pictures show a satisfactory take.

Heart Valve Grafts.—The heart valves removed from cadavers were obtained from the Cardiac Surgery Department of R. Y. L. Nair Hospital. They are further processed and freeze-dried. Difficulty was observed in maintaining sterility of final samples in view of the fact that these valves were removed during post-mortem, where satisfactory aseptic conditions cannot be maintained. The valves were then treated with ethylene oxide. However, contamination could not be avoided. Washing with solution containing Penicillin and Streptomycin was tried and sterile product was obtained by following this procedure in many cases.

Different conditions of freeze-drying were tried in each batch to observe the effect of the process on the condition of valves and to evolve a satisfactory routine for drying so as to maintain elasticity and satisfactory reconstituting properties.

8. *Plasma Fractionation—Fibrinogen.*—Fibrinogen is prepared as fraction I (Conn) from fresh plasma in small batches following the two donors method as described by Nitchman. Each batch of fibrinogen is tested for sterility and fibrinogen content. Estimations of the fibrinogen content in fraction I (clottable protein) are carried out and 60-65 per cent clottable protein is obtained which is satisfactory ; methods to purify this product further by following the methods described by H. Blomback are being developed.

The clinical use of this material has been encouraging. It has been used in cases of *post-partum* and post-operative haemorrhages. It has also been used with good response in a case of haemorrhage following snake bite. Laboratory tests in this case have shown satisfactory increase in fibrinogen level after administration of this fraction.

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

Gamma Globulin prepared from large pool of plasma is being used in prophylaxis of infective hepatitis and also for prevention of measles. It has also been used for various non-specific virus infections with encouraging results.

Antibody levels of each batch of Gamma Globulin against poliomyelitis, diphtheria and streptolysins are being studied.

The write-ups for printing of informative pamphlets for the various plasma fractions have been prepared and these are being printed. Follow-up cards have also been prepared for each product and will be sent with each supply of plasma products to enable us to collect follow-up data on the clinical use of this products.

(3) Department of Chemotherapy

1. *Synthesis of potential antitubercular agents.*—The work on and unsymmetrical thiocarbanilides reported in the earlier period was completed.

In view of the good antitubercular action reported for derivatives, about 45 new compounds having or 1, diphenylethyl moiety on one side and a tertiary nitrogen on other which, in some cases, may be a part of a heterocyclic ring system, have synthesised. The *in vitro* antitubercular screening of these compounds nearly 20 compounds out of this series to be active at 40 $\mu\text{g/ml.}$ of which 3 were active even at 5 $\mu\text{g/ml.}$

2. *Cytotoxic Substances.*—In continuation of previous work on bases of benzaldehyde nitrogen mustards, nearly 50 new Schiff bases and hydrazides were synthesised and characterized. Twenty-three compounds were prepared in large quantities and sent to Cancer National Service Centre, U.S.A., for screening. Reports for 30 of these in hand and it is found that in general these compounds are non-toxic. testing carried out against clinically predictable tumour systems in the animals has shown 4 of our Schiff bases to possess good order of activity. Two of compounds (N. S. C. 101485 and 101487) gave all cures in a group of 6 with Dunning Leukemia at 11.0 mg/kg. per day and 100 mg/kg. per day pectively. None of the test animals died. Further, a third Schiff base, (N.S.C. No. 94540) cured 4 rats in a group of 6 at 5.0 mg./kg./day in Leukemia with the death of one animal. Another compound, N. S. C. 1 has successfully passed the sequential screening in Lymphoid leukemia 121 (mouse) (a tumour of considerable clinical interest) and is being considered for further study by C. C. N. S. C. Among the hydrazides, one compound (N. S. C. 1014489) cured 4 rats in a group of 6 in Dunning Leukemia at mg/kg/day with no animal death.

3. *Psychotropic and C. N. S. active substances.*—The synthesis of bases from 3,4,5—trimethoxy acetophenone, benzyl phenyl ketones related derivatives was continued and 85 compounds in this class were prepared. According to the screening report from the C. S. I. R. Drug Screening Unit the Department of Pharmacology, Seth G. S. Medical College, 13 have shown promising results and are selected for further study for various pharmacological activities. Two compounds showed very promising activity as major tranquilizers.

4. *Synthetic Diuretics.*—In continuation of the previous work on s-triazines as possible diuretic agents, newer compounds with amino group at position 2 aniline or substituted aniline group at position 4 and mercapto, alkylmercapto, aralkylmercapto or hydroxyl group at position 6 of the triazine were synthesised. About 60 compounds of this type were prepared and majority of these were tested for diuretic property by the C. S. I. R. Unit at Seth G. S. Medical College. Some of the compounds showed substantial activity in rats. of these compounds having shown promise are taken up for further investigations.

Another series taken up for study was imidazol (1, 2-a) pyrimidine derivatives with 4-chloro-3-sulphamylphenyl group in position 2 and various substituents in positions 4 and 6. Among the compounds prepared, ten from this series were submitted for diuretic screening. No substantial activity was noted in these in the preliminary screening.

5. *Toxicological Studies—A. Manganese Poisoning.*—The work on investigation of hair manganese levels in mine workers was completed. It was found that the hair manganese level is a very good indicator of the degree of exposure to the metal. In view of the reported beneficial results of prophylactic oral calcium disodium EDTA administered in the manganese miners of Morocco, the study of its effect on the hair manganese levels in rabbits, is in progress.

B. *Degree on exposure to Parathion in workers handling the product during manufacture and packing.*—At the request of the Central Labour Institute, Government of India, Ministry of Labour, we have taken up the investigation on the presence of p-nitrophenol in the urine of exposed persons and simultaneous determinations of cholinesterase activity of blood (Plasma and R. B. C. separately) in collaboration with Dr. S. K. Chatterjee. The urinary p-nitrophenol, a metabolite of parathion, is being determined by colorimetric method, while the enzymatic activity is being done by Michel's method of change in pH. The results of work done in one factory have already shown much higher sensitivity of the p-nitrophenol determination in detecting the extent of exposure.

6. *Drug Screening Unit.*—93 compounds were screened against *M. tuberculosis* (H₃₇R_v) of which 30 showed activity in the range of 40 µg/ml and below. The 3 ethylenediamine derivatives, which were active below 5 µg/ml., are being taken up for further investigations.

136 compounds were screened against *Staph. aureus*, of which 17 were active at 30 µg/ml. and 19 at higher concentration in the range of 40-100 µg/ml.

136 compounds were screened against *Salmonella typhi*. Only 5 compounds showed activity at 40 µg/ml. while 26 showed moderate activity in the range of 100-200 µg/ml.

133 compounds were screened against *V. Cholerae* but only 4 were found to be active at 40 µg/ml.

78 compounds were tested for antifungal action. Only 3 were found active against *Candida albicans* (2 at 200 µg/ml. one at 100 µg/ml). Five compounds were active against *T. mentagrophytes* and 3 active against *T. rubrum*.

One thiazolyl thiocarbanilide synthesized earlier in the department and found to have high *in vitro* antitubercular activity (nearly that of I. N. H.) was tested for maintaining adequate blood level in guinea pigs. Whereas the sera of animal fed with diet containing I. N. H. at a level of 0.1 per cent showed total inhabitation from the 6th day of feeding, the thiocarbanilide under test reached tuberculostic concentration only after 20 days of feeding at the level of 0.3 per cent in diet.

7. *Micro-analytical Section*—Micro-analytical section carried out micro-analyses of 710 compounds for nitrogen and 130 compounds for carbon and hydrogen during the period under review.

(4) Department of Clinical Pathology

1. *Studies on Staphylococci and other organisms*.—The work for the studies on AntibioGram Pattern, phage typing and Serological Classification of pathogenic staphylococci was started in April 1964 and finished in August 1966.

Serological typing was done in detail by three different methods such as fluorescent antibody, agglutination method and gel diffusion techniques. Agglutination technique proved to be superior to the other two techniques. Phage typing proved to be useful. The work was submitted for the degree of Master of Science of the University of Bombay and was accepted.

An attempt to study this technique of Fluorescent Antibody on certain other organisms such as *V. cholerae* and *Leptospira* is being made. Since August 1966, immune sera against causative organisms under study have been prepared. The work is making progress.

2. *Studies on the Diagnosis of Enteric Fever*.—The aim of this study was to evolve a rapid, simple, specific and reliable test for the diagnosis of enteric fever. The limitations of the classical Widal Test are well recognised. Hence an attempt has been made to elucidate recent techniques, viz. Fluorescent antibody test and Haemagglutination Test in comparison with the routine laboratory test.

A total of 372 sera samples have been screened for the passive H. A. test. The sera were classified as follows :—

202 culturally proved enteric fever cases ;

90 normal cases (without any history of T. A. B. inoculation or infection) ;

40 T. A. B., inoculated cases, and

40 Non-enteric pyrexial cases.

Immunofluorescence test was carried out on a total of 262 sera samples including culturally proved cases, normal cases, inoculated cases and non-enteric pyrexial cases.

In addition 140 sera samples from normal individuals were screened for the normal agglutinin level.

The results of both the Immunofluorescence and H. A. tests were compared with the routine Widal and Culture Test.

The H. A. test proved to be more sensitive and reliable than the classical Widal Test. The results of the Fluorescent antibody test are quite comparable with those of the test.

3. *Serum Agglutinins following a T. A. B. inoculation.*—The study was taken up with a view to find out the period during which agglutinin 'O', 'H' and 'Vi' are detectable in the blood and may have a significance in interpretation of a single Widal Test done for diagnostic purposes. It may also indicate how often T. A. B. inoculations be carried out to ensure sufficient circulating antibodies in endemic areas like Bombay.

Subcutaneous injection of 1 ml. of a (formalized) T. A. B., vaccine was given to human adults in a single dose. A total of 374 cases was screened. Agglutinin response following vaccination could be well detected upto 2 months, if a titre of 1 : 50 is taken as the lower border line for definite agglutinin. By six months agglutinin response was practically nil except for P. A. H. Vaccination did not materially alter the 'Vi' antibody response.

In the absence of a method for detection of protective antibodies to judge the status of immunity, it would be desirable to repeat T. A. B. Vaccine every six months in endemic areas like Bombay. The work is completed and a paper has been published.

4. *Bacteriological Finding in Gastro-enteritis in infants.*—The above investigation was undertaken to elucidate the aetiology of gastro-enteritis in infants especially to enquire into the frequent association of enteropathogenic *E. coli*, with the syndrome. A combined project from the Institute was started by the Department of Clinical Pathology and Virology to study in detail the gastro-enteritis cases from both the bacteriological and viral points of view.

Bacteriological survey of 258 cases of acute gastro-enteritis in infants was carried out in this department.

E. coli was the predominant organism with an incidence of 74 per cent either present alone and/or in combination with other organism.

Presence of specific homologous agglutinins in the patient's sera was also looked for. The titres of agglutinins were insignificant.

Antibiogram pattern of the above isolated strains indicated that Neomycin was the most potent antibiotic.

The commonly isolated serotypes in order of their frequency were O26, O215, O111, O86 and O119. The work is still in progress.

5. *Immunofluorescent studies on V. cholera.*—The aim of the study is to evaluate the feasibility of the fluorescent antibody method for the early diagnosis of cholera from direct faecal samples.

Pursuant to this high titre sera of both Inaba and Ogawa strains were produced in rabbits and labelled with F. I. T. C. The conjugated serum showed bright fluorescence with the standard *V. cholera* strains. However, eight isolated strains so far tested showed weak fluorescence. Further standardisation of the test is in progress to improve the specificity of the test.

6. *Studies on Anonymous Mycobacteria isolated from human sources.*—A study of this problem was undertaken in the month of August, 1964 and is still being continued. A report on the aims of undertaking this problem, isolation of anonymous strains and their morphological, cultural and biochemical characters was made. This year, some additional work was carried out and is described below.

A total number of 67 strains of mycobacteria were studied, out of which 40 strains are anonymous mycobacteria, 18 strains are typical tubercle and the rest 9 included both standard anonymous and typical mycobacteria. All these strains were tested for their sensitivity for drugs like streptomycin, PAS, INH, Ethioanamide and Thioacetazone. They were also studied their pathogenicity to the various experimental laboratory animals guineapigs, mice and fowls. Special attention was paid to the strains isolated from the samples of C. S. F. collected from the cases of tuberculous in children. These strains showed peculiar characteristics and hence studied in detail. The results of the detailed study of these strains were ed to the 16th Annual Conference of Indian Association of in Ranchi in December 1966.

Antigens were also prepared from standard anonymous and and high titre sera obtained after immunizing rabbits. The obtained are used in the study of serological relationship either by tination test or by ESS test. HA test is already standardized. Since titre obtained in this case is very low, alternate method of immunizing with dried powder of bacilli was used. These sera will be tested by ESS We also intend to carry out some more pathogenicity tests in mice by intracerebral inoculation.

7. *A study of Leptospirosis.*—The work on the study of leptospirosis which commenced in June 1965 is being continued further. The purpose of study on leptospirosis has been to determine the reservoirs of infection and study the extent of infection in man and animals by cultural and methods.

Stray cases of leptospirosis are reported to us from time to time from hospitals as well as outstations. The methods employed for serological gations are the Sensitized Erythrocytes Lysis test and the Microscopic tination Tests.

Urine specimens and other pathological materials from suspected beings and animals, when available, are examined for the presence of spires by dark-field microscopy and the silver impregnation staining and attempts made to isolate these.

A total of about 190 human serum samples were tested for antibodies. Antibodies suggestive of recent or past infection with were detected in some of them,

About 120 sera from suspected and apparently healthy animals such as rats, cats, dogs, sheep, buffaloes-calves and bullocks were also tested for the evidence of leptospiral infection. In microscopic agglutination tests, only selected samples of sera were included for the study. Out of 21, 18 reacted with the leptospiral antigens.

Leptospire could not be detected in monkey kidneys and sewage samples. Five samples of each were thus examined.

A paper on 'A Serologic Survey of Human Leptospirosis in Bombay' was presented at the Annual Meeting of the Indian Association of Pathologists held at Ranchi in December 1966.

The above study is followed up.

Flourescent antibody technic is being tried for the early diagnosis. Anti-human-globilin conjugate is prepared for the detection of leptospirus by indirect method.

A few samples from sewers and animal house workers have been collected for the evidence of leptospiral infection. The methods employed for serological investigations are sensitized erythrocyte lysis tests and the microscopic agglutination tests. Samples are also collected from rats.

•Preliminary work is started to prepare vaccine against standard and local strains of leptospira. The work is in progress.

8. *Studies on Toxigenicity and Lysogenicity on diphtheriae*.—A comparative study of different diphtheriae strains for their toxinogenic and lysogenic properties has been commenced. The cultures are isolated on Hoyle's tellurite medium. Toxigenicity is determined by Gorman's method and lysogenicity by U. V. irradiation. The induced phage is tested by Adam's Overlay technique.

During the last four months of this study, 35 strains have been obtained with case histories. The study is in progress.

(5) Department of Entomology

1. *Studies on Musca sorbens*.—In the collection of allied species of Muscid flies in the compound of Haffkine Institute, it was observed that *Musca nebulo*, Fabr. and *Musca sorbens*, Wied. were the only Muscid flies collected, sharing the same habitat. Attempts were made to breed *Musca sorbens*, Wied. under laboratory conditions, and its biology studies. Successful breeding of *Musca sorbens*, Wied. was attained using sugar cane cubes and agar medium.

These two species could be differentiated morphologically and biologically on some external characters. However, in spite of these differences the tissue grafts of corpora allata from *Musca sorbens*, Wied. to *Musca nebulo*, Fabr. and vice versa readily took place, but there was no development of the ovaries in the allatetomised females of these species. The transplantation of *corpus allatum* in the same species effects the development of ovaries and fat metabolism, as was seen here in *Musca nebulo*, Fabr.

This fly is incriminated in the transmission of certain ailments. The obtained in the laboratory could enhance studies to see its efficacy in mission of diseases.

These studies throw important light on the physiological behaviour of species of flies.

2. *Mouth parts of fleas and their feeding mechanism.*—The significant point about the studies on the biting and feeding mechanism of fleas has shown that the proboscis bends in the tissue of the host. This is brought about the presence of a small muscle fibre in the lacinae. The difference in the feeding behaviour between *X. Cheopis* and *X. astia* lies in the fact that the latter settles down to feed straightaway while the former moves about for some time probing, piercing and withdrawing before it finally decides to feed at a particular spot. This behaviour for further studies may throw light on the mechanism of plague transmission.

3. *Breeding of two species of rats in the laboratory.*—*Tatera indica* from Wild was bred in the laboratory for five generations so far. It was noticed that if the same male is used for insemination, the gestation period is reduced by a number of days. *Rattus rattus* was bred only up to one generation. Attempts are being made to rear *Golunda Sp.*, *Tatera Sp.* and *Rattus mackenzii* collected from Gevrai and Daulatabad fort under the laboratory conditions.

These attempts will enable a worker to breed wild rodents for plague epidemiological studies in the laboratory.

4. *Testing of Insecticides.*—Laboratory trials were undertaken to evaluate, the efficacy of sevin; a bicarbamate insecticide, against fleas. In the field bait boxes were dusted with 5 per cent sevin dust and the fleas reduction observed. This is a new product in the range of insecticides and it is worth knowing its efficacy.

5. *Plague epidemiological studies in Marathwada region.*—These studies were carried out at 10 places in the districts of Osmanabad, Bhir and Aurangabad in the Marathwada region of Maharashtra State. The studies were started on 19th October 1966 and concluded on 27th January 1967.

In all, 2,324 rats were collected in these studies out of which 2,256 were *Rattus rattus* forming 96.6 per cent of the total catch. In addition to *Rattus rattus*, other species of rats were also collected. These were *M. musculus*, *B. bengalensis*, *R. blandfordi*, *L. booduga*, *T. indica*, *Golunda ellioti*. The fleas collected from these different species of rats are being identified.

Field trials with Zinc Phosphide in bait boxes dusted with sevin were carried out at Naldurg, Osmanabad, Murud, Ambejogai, Parli-Vaijanath, Pali, Bhir and Gevrai. The reduction in rat density as a result of these trials was significant. In all 453 dead rats were recovered. The majority of these rats belonged to *R. rattus* group. Field trials were also undertaken with an anticoagulant Rodenticide (Rodafarin at 0.025 per cent) at Naldurg and Osmanabad. A total of only 23 dead rats could be collected during these trials.

The point of interest in these series of pilot experiments was that at a number of places the baiting campaigns were undertaken after arousing the enthusiasm of local village population, and done by them. Secondly it shows that on an over all basis *R. rattus* predominates in the area and that trapping may be more useful in the houses.

6. *Studies on Susceptibility of Rattus rattus to plague.*—Rats collected from different places in Marathwada region are being challenged to a standard dose of *P. pestis* in order to assess their susceptibility status to plague. It has been observed that rats from Sholapur, Osmanabad and Murud show 67·5 per cent, 69·5 per cent and 65·5 per cent resistance respectively, to a standard dose of virulent *P. pestis*.

Rattus rattus from these areas are being tested in the laboratory to see any tolerance to Rodafarin. These studies are continuing.

7. *Studies on snakes salivary glands and venom output of snakes.*—Histological and morphological studies on the salivary glands of some non-poisonous snakes are being done to observe any differentiation in mucus and serrus secreting cells.

Pooled Russel's viper venom when centrifuged gives out an amount of debris at the base of tubes. Experiments were carried out to determine the toxicity of supernatant and the debris collected after centrifuging. The debris is non-toxic to mice, while the supernatant liquid is highly toxic. For immunological purposes now only the supernatant liquids are lyophilised and stored.

Preliminary trials done with Haffkine Institute Polyvalent Antivenin have shown that it gives protection in 20 gm. mice against 0·5 mg. of King Cobra, 0·3 mg. of Banded Krait and 0·4 mg. of Bamboo pit viper.

When completed these studies will throw light on the actual areas of cells that give the enzyme known as poison.

(6) Department of Immunology

1. *Isolation of Clostridia from soil and dung.*—All our previous attempts to isolate single colonies of the obligate anaerobe *Clostridium tetani*, which produces large amounts of tetanus toxin, using the usual thioglycollate medium, were not successful. Success was attained when a layer of thioglycollate agar was poured over the seeded surface of the same agar medium and incubating the petri dishes in anaerobic jars. It was also found that carbon dioxide atmosphere in the anaerobic jars gave better colony growth with less gas formation than an atmosphere of hydrogen.

Using the above technique, twelve strains of *Clostridia* were isolated from soil and dung. The differentiation of the *Clostridia* from other anaerobic organisms was possible by the use of ferrous citrate (120 mg. per cent) and sodium sulphite (60 mg. per cent) in the medium. The *Clostridia* were revealed by the dark colour of their colonies.

2. *Studies on Bacteriocines of Clostridia*.—The twelve strains of isolated as above were tested for the presence of phages or bacteriocines *Clostridium tetani* as the indicator strain. Bacteriocines are very spectrum antibiotics which are protein in nature and are induced because the presence of certain polynucleotides in the cytoplasm. The test was out by plating out the *Clostridia* on a lawn of the *Cl. tetani*. It was found two strains of *Clostridia* labelled C3 and C5 were producing haloes around them. The plaques were due to the formation of Bacteriocine and not due to the presence of phage. This was confirmed as follows: On U. V. induction and filtration, no plaques were formed when Adam's overlayer technique was used showing that the plaques were not due to phage. The culture showed antibiotic action as tested by agar diffusion technique in a plate with a lawn of *Cl. tetani* and the antibiotic activity was destroyed by papain to the culture filtrate. The bacteriocine was effective both on the sporulating and non-sporulating strains of *Cl. tetani*.

Attempts were then made to remove the Bacteriocine producing property by eliminating the cytoplasmic DNA responsible for its production. Prolonged irradiation with ultraviolet light, or use of different concentrations of acriflavine proved useless to remove the factor. Adding different concentrations of acriflavine to synchronous cultures (obtained by heating the cultures at 60° C for 15 mts., immediate cooling and subculturing) was also not effective. Plating out on agar medium containing different concentrations of acriflavine also did not help. Finally, the use of different concentrations of penicillin and 5 ug/ml. acriflavine showed that at concentrations of penicillin giving rise to spheroplasts, acriflavine was effective in eliminating the Bacteriocinic factor. These experiments were repeatedly confirmed. The bacteriocine-free culture of C3 is now being used for the study of the mode of infection.

It is proposed to study the nature of the bacteriocine and its mode of action.

3. *Fractionation and study of biologically important factors of Russell's Viper Venom*.—The Russell's viper venom was fractionated by gel filtration through sephadex G75. Elution was carried out with 0.05M tris-HCl buffer at PH 7.6 or with distilled water. The fractions obtained were examined for the following enzyme activities: Phospholipase A, phosphodiesterase, 5'-nucleotidase, phosphomonoesterase, protease, coagulase, anticoagulase and L-amino acid oxidase. The fractions were also examined for toxic and dermonecrotic actions. Purity and homogeneity of the fractions were checked by means of starch gel electrophoresis. The first fraction obtained with water as eluant contained phospholipase A activity and was almost free from other activities studied by us. The three phosphatidases, i.e. phosphodiesterase, 5'-nucleotidase and phosphomonoesterase overlap to a very large extent when separated with tris-HCl buffer and hence occur in the same fraction.

The toxic factor does not seem to possess any of the above mentioned enzyme activities and may require a cofactor to show its activity. The molecular weight of the toxic factor is comparatively low as judged by its elution volume after gel filtration.

Comparative study of white and yellow venoms from different snakes of the Russell's viper species showed that white venom did not contain proteolytic enzyme while L-amino acid oxidase and phospholipase A contents were much less. The yellow venom showed strong dermonecrotic activity while the white venom showed negligible activity. The dermonecrotic activity was traced to the presence of proteolytic activity in the separated fractions. Fractionation of the R. V. Venom is now being carried out on a preparative scale in a column of 3.4 cm. diameter and 65 cm. in length packed with Sephadex G-150. 0.5M NaCl is used as the eluant. It may be possible to market the phospholipase A after purification and standardization since this seems to have some demand from research workers in India and abroad.

4. *Study of factors responsible for production and assay of protective antigen of pertussis vaccine.*—The variability in the assay values of the same pertussis vaccine reported by different laboratories prompted us to study the factors chiefly concerned with the measurement of potency of pertussis vaccine. The M. R. C. trials have now confirmed that the laboratory method using intracerebral challenge of mice immunized fifteen days earlier with dilutions of vaccine injected intraperitoneally is the best method of assay. However, it was found by us that the strain of mice used is very important for this assay. The following strains of mice were tested : (i) Haffkine strain, (ii) Swiss-Haffkine strain, (iii) Kasauli strain, (iv) Strain from Lucknow, (v) NIH general purpose strain. The results showed that Kasauli strain gave uniform and consistent results. This strain was also more susceptible to histamine challenge after injection of pertussis vaccine.

The next step was the choice of a suitable challenge strain. Using NIH standard vaccine as reference and other vaccines for comparison, consistent results were obtained only when the virulent challenge strain from Kasauli (2-Atox) was used. The challenge strain 9797 did not give consistent results.

Using the Kasauli strain of mice and 2-Atox as challenge strain, about 24 strains of *B. pertussis* were tested for their ability to produce adequate amounts of protective antigen. The vaccines were prepared by the shaken culture method in Cohen-Wheeler medium containing acetate and incubated at 35° C for 24 to 48 hours. The cultures were killed by adding 0.01 per cent merthiolate and centrifuged to remove the culture filtrate. The cells were suspended in 0.01 per cent merthiolated saline and heated at 56° C for 30 minutes and cooled. The vaccines were allowed to stand in cold for about a month before testing their potency, using NIH standard vaccine as reference. Results showed that strain 134, 1709 and 1855 were superior to the others.

The vaccines prepared with these strains have been now tested by MRC Laboratories in England, NIH Laboratories in the United States and the Central Research Institute, Kasauli and all have pronounced them as better than the required standards.

5. *Nutritional requirements of Bordetella pertussis.*—The study of the nutritional requirements of *B. pertussis* is of great importance in the production of whooping cough vaccine.

Using acid hydrolysed casein medium of Cohen and Wheeler, attempts were made to stimulate growth using various hexoses, pentoses and intermediates of tricarboxylic acid cycle. In 24 hours of growth at 35° C in a rotary shaker, it was found that the organism could not utilize hexoses as a source of energy. Of all the other compounds, only acetate showed stimulation of growth in a concentration of 0.02 g per cent.

The requirements of vitamins, amino acids, purines and pyrimidines were then studied using "all minus one" technique. Results showed that the organism does not need any vitamins, purines or pyrimidines. Good growth was obtained in a medium containing the four amino acids, viz. cystine, proline, alanine and glutamic acid. In this medium growth was equal to the complete 19 amino acid medium or Cohen-Wheeler medium. In the 4 amino acid medium the organism does not need starch which is used in Cohen-Wheeler medium to absorb the toxic factors formed during the growth of the organism.

6. *Nature of the protective antigen of B. pertussis.*—The pertussis organism, though one of the smallest of bacteria, has a complex antigenic make-up consisting of protective antigen, histamine sensitizing factor (HSF), agglutinin, hemagglutinin and thermolabile and thermostable toxins.

The hemagglutinin and thermolabile toxins are present mostly in the culture filtrate. The agglutinin can be extracted from the organism by washing. The protective antigen and the HSF are firmly bound to the cell wall and could be solubilized only by sonication of the washed cells. Most of the previous workers have found that the HSF activity cannot be separated from the protective activity and therefore, they believe the two activities are the manifestations of the same substance. Attempts were made to see whether it is possible to remove or reduce the HSF activity without affecting the protective activity and also to find the nature of the protective antigen.

The sonicate of washed cells of *B. pertussis* was treated with various enzymes, such as trypsin, chymotrypsin; muscle phosphorylase, alkaline phosphatase from *E. coli*, ribonuclease, phospholipase C from *Cl. welchii* and egg white lysozyme. Results showed that the proteolytic enzymes destroyed both activities and lysozyme had no action on either. The phosphatase, phosphorylase, ribonuclease and phospholipase C destroyed HSF activity but not protective activity. Acetone and alcohol treatment destroyed both HSF and protective activity. From these results, it may be speculated that the protective antigen is a protein which is linked to a lipid through a phosphate ester bond. The complete molecule only shows the HSF activity. Further experiments are being carried out to verify the above concept of the nature of the protective antigen and the histamine sensitizing factor.

7. *Studies in structure of Immunoglobulins of horse serum.*—The distribution of antibody activity in the different electrophoretically separable fractions of hyperimmunized horse serum was studied. About 90 per cent. antibody activity was present in the beta globulin region, 8.5 per cent. in gamma globulin region and only about 1.5 per cent. in the alpha globulin region.

Immunologically pure antibody globulin IgG was prepared by precipitating antisera at 0.33 saturation followed by chromatography on DEAE cellulose.

Immunologically pure Immunoglobulin IgA was prepared by a preliminary fractionation using zinc sulphate precipitation followed by preparative electrophoresis on agar gel.

Peptic digestion of IgG and IgA showed that only one fragment from each, both having gamma mobility and having antibody could be isolated. These fragments cross-reacted when tested by Ouchterlony test with their parent immunoglobulins, but showed reaction of identity among themselves. This indicates that the pepsin fragment (Fab) of both IgG and IgA may be alike probably due to identity of the light chains and Fd fragments in both immunoglobulins.

Papain digestion of horse IgG and IgA revealed that two fragments are formed from each one of them. Fab fragment from IgG showed reaction of identity with its counter part from IgA and the other FC fragments of the two immunoglobulins were dissimilar. Further work is continuing on the isolation of the fragments and the chains and investigations of their structure.

8. *Studies in properties of horse immunoglobulins.*—When an animal is immunized with a pure antigen the antibodies produced are very heterogeneous. In human beings, so far three important classes of antibodies have been identified with differing electrophoretic mobility, molecular weights etc. and also differing in immunological properties, such as complement fixation, anti-complementary activity, skin ligation, transplacental transmission, transmammmary transmission to milk etc. It is proposed to purify the immunoglobulins in horse and study their properties. Purification of IgG and IgA has already been carried out. Our preliminary work has indicated that there are more than 3 classes of antibodies in horse serum. We have been getting a small amount of antibody activity in the alpha 2 fraction. Moreover, by the use of rabbit antifloccule sera 4 to 5 lines were seen in immunoelectrophoresis. The rabbit antifloccule sera was prepared using washed floccules of diphtheria toxin and antitoxin which should, therefore, react with only the immunoglobulins of horse sera. The number of immunoglobulins can also be confirmed by absorbing them with anti-light chain serum since it is known that the light chains are common to all the immunoglobulins.

9. *Anaphylactogenic antibodies in horse.*—The processes leading to tissue damage involve the interaction between the antigen (or allergen) and its specific antibody which initiate the liberation of pharmacologically active substances which cause the anaphylactic symptoms. It is also known that the valence of the antigen is important for this purpose. The passive cutaneous anaphylaxis reaction in the guinea pig was, therefore, taken up for study using undegraded and enzyme degraded antihorse sera. It was proposed to separate the important immunoglobulins and then take up this study. Horse IgG and IgA have been purified and they have been degraded with pepsin and papain. The fragments have been separated. Fragmentation using mercaptoethanol or cyanogen bromide will also be prepared for testing. However, due to shortage of guinea pigs in recent months, this work could not be pursued with vigour.

10. *Simultaneous active and passive immunization against tetanus.*— This work was sponsored by the Department of Technical Co-operation, London, who bore about 80 percent of the total cost of Rs. 49,106 and the Government of Maharashtra who bore the remaining expenses. The object of this work was to find out the best method of prevention of tetanus among unimmunized patients with tetanus prone injuries. In such patients, it is known that 1500 IU or even 5000 IU of antitetanus serum gives temporary protection for a week or two only. The antiserum also interferes and prevents active immunization with the fluid tetanus toxoid if it is injected at the same time. Immunization with fluid toxoid alone in such patients is risky because the patients cannot be considered to have been actively immunized with adequate protective antibodies unless they receive two injections at an interval of 4 to 6 weeks. However, it has recently been reported that adsorbed tetanus toxoid given at the same time as 1500 units of antiserum can give adequate short and long term protection. We wished to compare the antibody titres in 3 groups of patients : one group (schedule A) received 1500 IU of tetanus antitoxin in one arm and in the other arm 0·5 ml (20 Lfs.) of adsorbed tetanus toxoid; the second group (Schedule B) received 5000 IU of tetanus antitoxin and 0·5 ml. (20 Lf) of adsorbed tetanus toxoid; the third group (Schedule C) received only tetanus toxoid. All the patients received a second injection of 0·5 ml. (20 Lf) toxoid 4 to 6 weeks after the first injection. There were 7,274 patients in schedule A, 7,020 patients in schedule B and 1,561 patients in schedule C making a total of 15,855 patients. Serum samples were collected before the first injection, at the time of second injection of toxoid and a third sample 6 weeks to 1 year after the first injection. Altogether, there were 596 samples of sera. The immunizations were carried out at the J.J. Hospital under the supervision of Dr. B. J. Vakil and the sera were analysed at the Haffkine Institute. The sera and tetanus toxoid were products of the Haffkine Institute. Dr. J.W.G. Smith of the School of Tropical Medicine, London, kindly verified the antibody titres of some sera from the patients.

There was no tetanus in any of the immunized patients during the one-year period of observation.

About 40 percent of the patients had a basal immunity of 0·01 IU/ml or more. Only one out of 148 analysed about 4 to 6 weeks after the 1st injection in the three schedules showed less than the protective level (0·01 IU/ml). The level of antibodies rose sharply after the second injection of toxoid and remained above the protective level for about a year in practically all the patients. The titre of antibodies in the sera of patients in schedule A and C showed no statistical difference. The antitoxin level in schedule B was less than the other two schedules in the early period but tended to be not significant after 120 days.

This study shows that in patients with tetanus prone injuries, simultaneous active and passive immunization with 1500 or 5000 IU of antitetanus serum and 0·5 ml of adsorbed tetanus toxoid (injected at the same time in a different region), is a practicable procedure in India where most of the population is not actively immunized.

11. *Active immunization in the pregnant women with fluid tetanus toxoid.*—The aim of this work was to establish a quick and adequate immunity for the mother against puerperal tetanus and the newborn against neonatal tetanus. The work was carried out in collaboration with Dr. A. F. Heredia.

Pregnant women attending the antenatal clinic of J. J. Hospital usually in their 7th or 8th month were given 3 injections of 1 ml. each of Haffkine Institute fluid tetanus toxoid at intervals of 15 days. Eighty-three samples of mother's blood and 23 cord blood samples were analysed. All the blood samples from the mother and from the cord after 3 injections of toxoid showed a titre of over 0.01 unit per ml. of antitoxin. Maximum immunity was found after 3 injections, the last one given 3-4 weeks before the date of delivery. It is, therefore, concluded that 3 doses of fluid toxoid at intervals of 2 weeks would ensure adequate protection against tetanus for both the mother and child.

12. *Immunological investigations in lens proteins.*—Previous work in our laboratory showed that the alpha crystallin of lens proteins is the organ specific antigen and has proteolytic activity. The insoluble albuminoid of lens which increases with age and is very high (over 70 percent) in cataractous lens was found to be a polymer of the soluble alpha crystallin. It was, therefore, of interest to investigate the relationship between alpha crystallin and the albuminoid. The lens of rats and rabbits at different ages from birth to one year were analysed for total proteins, soluble proteins, albuminoid and alpha crystallin. A reciprocal relation was found between alpha crystallin which decreased with age and albuminoid which increased with age.

An application for grant-in-aid is made under PL480 Scheme.

(7) Department of Nutrition and Biochemistry

1. *The nucleotide (Base) Composition of the RNA of the Salmonella Organism.*—The separation of nucleotides by high voltage-paper electrophoresis was standardised using carbon tetrachloride as the cooling agent in the system. The Ph of the buffer seems to be critical for the satisfactory separation of the nucleotides. At pH 3.2 the four nucleotides separate in the following order Cytidylic (C), adenylic (A), guanylic (G) and uridylic (U) acids. The first 2 have very close R_f values.

The base composition of the organisms *S. Typhi*, *S. paratyphi A* and *S. paratyphi B* grown in nutrient broth and synthetic medium was determined by the above procedure using the RNA hydrolysates. Pure RNA was isolated from sonicated cells using Cetyl tri methyl ammonium bromide as the precipitating agent. The results are given in the tables.

Percentage of Nucleotides (Moles Percent)

Organism	Medium	C	A	G	U	A+G	C+U	A+G C+U
<i>S. typhi</i>	N. broth	26	20.5	31	22.5	51.5	48.5	1.06
	synthetic	25	22	30	22.7	52	48.5	1.09
<i>S. Paratyphi A</i>	N. broth	26	24.5	27.5	22	52	48	1.08
	synthetic	23.5	28.5	25.5	22.5	52	48	1.08
<i>S. para typhi B</i>	N. broth	28	20	31	20	51	47.5	1.07
	synthetic	30	15.8	36.5	17	51	47.8	1.07

It is seen that the Purine—pyrimidine ratio is more or less same for all the 3 organisms, though the percentage of individual nucleotides varies. Not much difference was observed with Pu/Py ratio of cells grown in Nutrient broth and in synthetic medium.

2. *RNA Composition of the 3 Salmonella Organisms.*—Pure RNA from the 3 organisms were fractionated using methyl albumin coated on Kieselguhr—into soluble RNA and ribosomal RNA (16S and 23S) and the fractions were collected by sodium chloride gradient elution. As seen from the table the ribosomal RNA constitutes a major part of the Ribonucleic Acid in the cells (about 60-70%) in all the 3 organisms. However, a difference is seen in the RNA sub-fractions.

Percentage of RNA subfractions (cells grown in synthetic medium)

Organism		Soluble RNA	RNA-DNA Hybrid	Ribosomal RNA			
				Total	16 S	23 S	
(Percentage)							
S. Typhi	..	..	15.0	24.0	61.0	25.0	36.0
S. Paratyphi-A	..	..	24.5	5.3	70.5	12.5	58.0
S. Paratyphi-B	..	..	16.6	10.1	74.0	32.5	41.5

3. *A comparison of the synthetic ability of the Salmonella Organism.*—species variations in micro organisms is characterised by specific protein synthesis. The species differ in their constitution mainly due to the different enzyme make-up (protein) which is controlled by the genes (DNA) through different ribonucleic acids. The first stage of protein synthesis involves the activation of the amino acids in the presence of activating enzymes. The activated amino acids are bound to S. RNA, forming amino acyl S. RNA which are transferred to ribosomes where the peptide chain is formed. The messenger RNA which acts as the carrier of genetic information from DNA, gets attached to the ribosomal particles and directs the ordered assembly of amino acids into certain specific structures of the protein.

Since the nucleic acids play a major role in protein synthesis, studies on the synthetic ability (protein and nucleic acid synthesis) can lead to a better understanding of the fundamental difference in micro-organisms. The nucleic acid synthesis was studied using adenine and guanine as precursors. The three organisms were grown for 24 hours under shaken conditions at 37° C and a detailed study was made. After incorporation of labelled adenine and guanine, the cells were fractionated into acid soluble nucleotides, RNA and DNA. The total and specific activity in the above fractions were determined to get a comparative idea of the nucleic acid synthesis.

TABLE

*Incorporation of Adenine (A) and Guanine (G) into
the Cells and Individual Fractions*

Organism	Purine	Cells		Acid Soluble Fraction RADIO ACTIVITY			RNA	DNA	
		Total	Sp.	Total	Sp.	Total	Sp.	Total	Sp.
<i>S. typhi</i> ..	A	46	153	3.3	15,300	20.5	15,000	8	6,800
	G	46	143	2.4	8,400	16.8	12,200	8.3	8,000
<i>S. paratyphi A</i>	A	17	94	4.7	16,000	11	12,700	4.6	5,900
	G	17	104	1.8	4,600	13.3	15,100	5.7	8,675
<i>S. paratyphi B</i>	A	68	186	3.8	15,400	24.5	17,800	14.2	11,000
	G	60	180	3.2	12,400	21	16,000	9.7	9,300

Total activity—Per cent of initial activity.

Special activity—Counts per minute per 1 Unit of O. D.

Maximum utilisation of these precursors was observed in *S. paratyphi B* and minimum in *paratyphi A*, both in the whole cell and individual nucleic acid fractions. Since the total incorporation as well as the specific activity in the nucleic acids were found to differ among the 3 strains it is planned to study the RNA synthesis in detail in the sub-fractions—soluble and ribosomal RNA (16S and 23S).

4. *Glucose metabolism in Salmonella Organisms.*—A comparison of the metabolism of first and sixth carbon atom of glucose by *S. typhosa* and *S. paratyphi A* was made at two different growth conditions—stationary and shaking. The oxidation of carbon-1 of glucose involves the 'pentose pathway' (while carbon-6 goes through the EMP pathway and the resulting pyruvate getting oxidised through the Kreb's cycle. The ratio G-1/G-6 indicates the major pathway under operation. It was observed that under stationary conditions the ratio is 1.83 and 1.37 for *S. typhosa* and *S. paratyphi A* which is practically reversed under shaken conditions 1.48 and 1.85. It is also further observed that C1 and C6 enter into almost all the cellular macromolecules. Further work is in progress.

5. *Studies on RNA containing bacteriophage.*—Bacteriophage F4 was prepared from *E. coli* cultures by Ammonium Sulphate fractionation and cesium chloride gradient centrifugation. The RNA isolated from this phage was fractionated into sub-fractions. This, however, yielded only one peak corresponding to 14S position. Since this RNA is known to have messenger activity, its occurrence at 14S position is justified. Base composition studies are in progress.

6. *Dietary fat and Cholesterol esterase activity in the tissues of* of the plasma cholesterol circulates in the esterified form. The sites mechanisms of synthesis and hydrolysis of cholesterol esters in the not been well established. The esterification reaction taking place in serum is due to a transferase which does not require any Co-factor for activity. This enzyme has a specificity which is characteristic of the pattern of plasma cholesterol esters of the species. Liver is suggested as probable source of the plasma enzymes. This study was undertaken to the effect of dietary fat on the esterase activity of the organs.

Groups of 6 rats were fed safflower, coconut, groundnut, oils and at 20 per cent level in the diet for 6 weeks. The esterase activity in liver, intestines and pancreas were determined.

The absolute amount of newly esterified cholesterol in the plasma was significantly elevated in the coconut oil and vanaspati groups. in plasma free-cholesterol was parallel to the decrease in phospholipids. EF deficiency induced by a high saturated fat or a fat-free diet resulted in cholesterol esterification activity in liver *in vitro*.

The intestinal and pancreatic enzymes seem to esterify preferentially with unsaturated fatty acids. Different dietary fats did not any significant change in the esterifying capacities of both the intestine pancreas. The absolute values for the pancreatic enzymes were very higher than those for the intestinal enzymes.

7. *A critical study on the estimation of Serum Transaminases.*—The need following a uniform procedure for the colorimetric estimation of SGOT and SGPT in the clinical laboratories.

A uniform procedure and standard conditions with reference to the temperature and time of incubation and substrate concentration are recommended for the colorimetric assay of SGOT and SGPT in the clinical laboratory.

A substrate concentration of at least 4 μ moles of alpha-ketoglutaric acid to be used for 0.1 ml. of serum sample at 37° C for 1 hour. A high tition of Dinitro phenyl hydrazine (DNPH) is required for the optimum of the pyruvic acid hydrazone in the presence of the excess of the Toluene extraction is necessary to selectively separate the pyruvic acid from the mixture before developing the final colour with alkali. For activities, higher than 1,000 pyruvic acid units/ml/hr., it is preferable to the serum five or ten-fold. The enzyme activity should be expressed to the International Unit system in terms of micrograms of pyruvic acid/ml/hour or micromoles of pyruvic acid per minute per litre of serum, so that the results can be interpreted uniformly by the clinicians. Under the conditions mended, the SGOT Values of normal individuals are within a range of 80-160 μ g of pyruvic acid/ml/hr.

A similar procedure has been standardised for the assay of SGPT alpha-ketoglutaric acid concentration of 4 μ moles and DL-alanine of 40 moles. The average normal values for SGPT range from 60-100 pyruvic units/ml/hr.

8. *Protein metabolism with special reference to Vitamin B₁₂ and pantothenic acid.*—The metabolic interrelationships between the two vitamins and their effects on growth, nitrogen metabolism, blood constituents and liver nucleic acids were reported previously.

(a) *Distribution of the vitamins in the particulate fractions of liver in rat.*—Vitamin B₁₂ and pantothenic acid content of particulate fractions showed that the percent distribution of vitamin B₁₂ and pantothenic acid was more in the supernatant fraction as compared to that in nuclear and mitochondrial fractions. The oral administration of vitamin B₁₂ and pantothenic acid resulted in the increased concentration of the respective vitamins in the supernatant fraction of the liver.

(b) *Protein content of particulate fractions of liver.*—It was observed that vitamin B₁₂ or pantothenic acid when added to pantothenic acid deficient or vitamin B₁₂ deficient diet, respectively increases significantly the protein content of the supernatant fraction of liver. It seems, therefore, that the effect of either of the vitamins on the protein content of supernatant fraction is produced by the supporting action of the other vitamin. The stimulatory action of pantothenic acid on liver protein formation may have been mediated indirectly through its bound form (CoA) which activates the amino acids. The action of Vitamin B₁₂ on liver protein might have mediated through B₁₂ enzyme which acts at the step between activation and transfer of amino acids to ribosomal RNA as postulated by Johnson and his associates or it may be through its effect on nucleic acid synthesis. The latter hypothesis seems to be more feasible as it could be seen from the results reported previously that Vitamin B₁₂ increases the RNA content of supernatant fraction of liver homogenate.

9. *Studies in E. coli metabolism.*—Studies in various aspects of metabolic inter-relationship between Vitamin B₁₂ and pantothenic acid in rat were reported previously. This was extended to *E. coli* metabolism to throw more light on some of the aspects of metabolic inter-relationship between these two vitamins found in rat. Two strains 1. wild strain ; 2 Mutant 113-3 (Vitamin B₁₂/ methionine auxotroph) were employed for this purpose.

The effect of addition of pantothenic acid to the liquid medium of Davis and Mingoli on the growth of these two strains, was investigated. Pantothenic acid was found to have no effect on the growth of these organisms.

Further, the composition of the cell-mass was investigated. The organisms were grown on the liquid medium of Davis and Mingoli (Wild strain) and the same medium supplemented with methionine (mutant 113-3 strain). The cells were centrifuged and washed with sterile saline and finally dried in vacuum. The dried cell-mass was taken for the analysis of nucleic acids (RNA and DNA), pantothenic acid, Vitamin B₁₂ and amino acids. Further work is in progress.

10. *Microbiological methods for evaluation of protein.*—(a) It was reported last year that 7 different foodstuffs were assayed for protein quality by using protozoon *Tetrahymena pyriformis* W. The work was continued further and

more foodstuffs, viz. Leaf-protein (CFTRI), Bengal Gram, Green Gram, and Groundnut flour (Tata) were assayed for protein quality. Leaf-protein gave the highest value and was followed by Bengal gram, Groundnut flour and Green gram in order of protein quality.

(b) *Supplementation of foodstuffs with pure amino acids.*—It was reported previously that Kidney beans, red variety (*phaseolus vulgaris*) and Cluster beans (*Cyamopsis tetragonoloba*) are deficient in methionine and tryptophane. Hence the effect of tryptophane-methionine supplementation on the protein quality of these foodstuffs was investigated by assay with *Tetrahymena pyriformis* W. A significant rise in nutritive value of these foodstuffs was found due to methionine tryptophane supplementation.

(c) *Availability of amino acids.*—The organism *Streptococcus zymogenes* NCDO 592 is vigorously proteolytic and requires methionine, tryptophane, leucine, isoecine, histidine, valine and arginine for its growth. This organism was, therefore, used for estimating the availability of the above 7 essential amino acids in black gram, red gram, kidney bean (red variety), gowar, bajra, groundnut flour, and rajgira (*Amaranthus paniculatus*). Total amino acids were determined on the complete hydrolysate obtained by the action of mineral acid and alkali, while available amino acids were determined on the partial hydrolysate obtained by the action of papain at pH. 7.2 and 56°C for two hrs. The results are under process.

11. *Use of groundnut oil cake as a substitute for skim milk powder in the diet of stock animals.*—Groundnut oil cake flour is a wholesome food and contains 50 per cent. protein. It serves as a useful supplement to protein deficient Indian diets. It is also cheaper than animal protein foods such as milk, meat, etc. It was therefore, thought worthwhile to study the effect of groundnut oil cake as substitute for skim milk powder in the stock diet and on the amino acids content of the two diets.

The stock diet contains jowar, wheat, bengal gram, groundnut oil, yeast, calcium carbonate, sodium chloride and vitamins A and D, besides 20 parts of skim milk powder. The skim milk powder in the above stock diet was replaced by 20 parts of groundnut oil cake. The amino acids content of the two diets were determined microbiologically. The result showed that groundnut oil cake diet contains less lysine and methionine than the skim milk powder-diet. There was no difference in other essential amino acids content of the two diets. The growth response in rat was similar in respect of both the diets.

(b) *Use of ground-nut oil cake hydrolysate as growth medium.*—Groundnut oil cake hydrolysate with 9 per cent fat was hydrolysed (acid hydrolysis) and tried as a growth medium for *V. cholerae*, *S. typhosa* and *S. paratyphi* A. Better growth could be obtained for all those organisms under stationary and shaken conditions as compared to casein hydrolysate medium. A detailed will be

(8) Department of Pharmacology

1. *Studies on Antidiabetic Drugs*.—Five plant extracts were tested for their activity after oral administration to fasting rabbits. In our study, we had reported on 16 synthetic compounds. The present deals with the studies of various extracts of five plants, viz. IDR-7, IDR-9, IDR-10 and IDRN. Solvents for extracts were petroleum ether, and water. Water Extracts of IDR-8, at the end of 5 hours blood sugar to the extent of 24 per cent (average of 6 animals). results obtained with alcoholic and petroleum ether extracts were found to comparatively less potent. Similar results were obtained with water extract of IDR-9. As in case of IDR-8, alcohol and petroleum ether extracts gave less reduction in blood sugar. The other drugs tested had no significant antidiabetic activity.

2. *Studies on Amoebiasis—Amoebicidal activity of indigenous drugs*. Antiamoebic activity of the drug was tested both 'in vitro' and 'in vivo'.

In vitro, the drug was added to the Pavlova's medium which was used for growth of *Entamoeba histolytica*. After 45 hours incubation the growth observed and compared with that in the control tubes.

In vivo it was tested on golden hamsters infected with *E. histolytica* and albino rats infected intracaecally. The drugs were orally. At autopsy the degree of infection was compared with of the animals in the control group.

The drugs tested include IDR-2, IDR-6, IDR-7, IDR-8 and IDR-N. Out these IDR-2 and IDR-N showed activity only *in vitro* at 10 mg/ml. completely the growth. *In vivo* only IDR-6 was active at 25 mg/100 g. body wt. in hamsters and 10 mg/100 g. body wt. in albino rats.

The other drugs tested were guttiferin, B-guttiferin, -guttiferins and morellin from the plant known as *Garcinia morella*. These drugs did not show any results.

3. *Studies on indigenous drugs—(a) Isolation and characterisation of some principles IDR-5*.—A well-known plant used in *Ayurvedic* medicinal against bronchitis, asthma etc., was studied to obtain mainly the six constituents—(i) Essential oil (IDR-5a), (ii) Steroid like compound melting point of 111°C (IDR-5b), (iii) Fixed oil, (iv) A solid alkaloid and (v) A viscous alkaloid (IDR-5d) and (vi) A glycoside of high point. All these fractions were undertaken to study chemically in Essential oil was distilled under reduced pressure to obtain the following :—

(A) B. P. 140-160°/2 m. m., (B) B. P. 160°/2 m. m., (C) B. P. 160-200 /2 m. m. ;

16.6 g. of the essential oil was taken and distilled under reduced pressure. All the fractions were colourless liquids. These fractions were insoluble in water, and soluble in almost all organic solvents. Dilute solutions of the fractions (a) and (b) in alcohol decolourise dilute solutions of iodine and bromine indicating the unsaturation in the molecule.

3. (b) *IDR-7*.—This plant was extracted with alcohol. After complete removal of alcohol under reduced pressure, it yielded reddish coloured solid which was soluble in water. The aqueous solution of this crude solid indicated presence of alkaloid, sugars and glycoside. The solution of this crude solid when tested for its antibronchial and antidiabetic activity, gave encouraging results. By following paper chromatography, it was ascertained that the plant contained small quantities of an alkaloid and a glycoside and a large amount of sugar. By column chromatography using silica gel and activated alumina, we were able to isolate a glycoside in fairly pure state and an alkaloid in slightly impure state. When these purified fractions were tested for their antibronchial activity, they showed mild action.

(c) *CDR-7*.—The plant powder was first extracted with petroleum ether to obtain essential oil. The powder obtained after extraction with petroleum ether, was re-extracted with rectified spirit by percolation method. After removal of rectified spirit, the mass was dissolved in water and worked up for isolation of sugars and glycoside (mixture) by following the standard lead acetate procedure.

Pharmacological Tests.—The essential and total oils were found to be hypotensive in normal and atropinised cats. The respiration was also diminished. The alcoholic extract in dose of 0.02 mg. and the essential (1 : 20 dilution) in dose of 0.03 ml. were found to contract the ileum. The alcoholic extract, essential oil and total oil produced of guineapig's tracheal chain, while essential oil and total oil in doses of 0.01 and 0.005 ml. were effective in producing broncho-constriction in In acute toxicity tests done in albino mice, the alcoholic extract gave 100 per cent mortality with 2.5 g/kg. ; orally 2.5 g/kg. produced no death, but 10 g/kg. killed 20 per cent of the animals. The essential oil in 0.1 ml/mouse (18-20 g.) orally produced 100 per cent mortality and 0.0125 ml/mouse produced no death. Some antidiuretic effect was observed in rats.

Adhatoda Vasica.—The following preparation of *vasaka* and the alkaloid were tested for general pharmacological activities with special to their effect on bronchial musculature, preliminary toxicity and activity. This drug has the reputation of being effective in *bronchial Vasaka crude hydrochloride* (37/65) and *Vasaka hydrochloride* (38/65) not produce any appreciable effect on bronchial musculature. *Vasaka soluble pigment* (39/65) produced broncho-constriction in intact guinea *Vasicimone hydrochloride* (6/66) on the other hand produced but did not affect the broncho-constriction effect of histamine. On isolated preparations of guineapigs tracheal chain it produced contraction of the musculature. Alkaloidal fraction of *Adhatoda vasica* produced constriction effect on intact guinea pigs, and also on the isolated chain of guineapigs. This substance did not show much action in moderate amount. The maximum tolerated dose was 100 mg/kg. orally, 25 mg/kg. intraperitoneally and 5 mg/kg. intravenously in mice.

(d) *Solanum xanthocarpum* (CDR-1).—The glyco-alkaloid isolated from plant material has hypotensive properties. In a dose of 0.5 mg/kg. in cats, it produced marked fall of blood pressure. Compared to reserpine, it needs one fifty of the dose to have the above effect. The drug has slight antidiuretic properties too. The maximum tolerated dose in mouse was 100 mg/kg. orally, 25 mg/kg. intraperitoneally and 5 mg/kg. intravenously. The extract, however, had no antibacterial activity when tested by the filter paper disc method.

3(e) *Antimicrobial Activity of Extracts of Medicinal Plants*.—The study of the antimicrobial activity of plants reported as effective in the treatment of various microbial infections, in indigenous medicinal systems, was continued. Solvent extracts prepared from selected plant species, viz. CDR-9, CDR-11, to 16, CDR-18 and CDR-19 and IDR-11 to 14, were screened against *Salmonella typhosa*, *Vibrio cholerae*, *Escherichia coli*, *Shigella dysenteriae*, and *Staphylococcus aureus*. Of these the ether extract of CDR-9 and CDR-11 inhibited all five test organisms. The alcohol extract of CDR-9, the ether extracts of CDR-16 and CDR-19 and chloroform extract of CDR-12 inhibited four of the five test organisms and the alcohol extracts of CDR-14 inhibited three of the five test organisms.

The ether extract of CDR-16 also inhibited the growth of *Trichophyton mentagrophytes* and *Mycobacterium phlei*. An essential oil from a plant CDR-7 inhibited the growth of *V. cholerae* and *M. phlei*, was found ineffective in the treatment of experimental cholera in infant rabbits.

Several crude and pure compounds from *Adathoda vasica*, *Holarrhena antidysenterica*, and *Mesua ferrea* were tested for their antibacterial action. Vasaka crude hydrochloride, vasaka hydrochloride, vasaka water soluble pigment, kurchi crude and pure hydrochloride, all inhibited *Shigella dysenteriae*, *varshiga* in 0.5 per cent concentration.

Kurchi crude and pure hydrochloride also inhibited *S. aureus*, *S. typhosa*, *V. cholerae*, *E. coli* and *S. dysenteriae* vars shiga, sonnei and flexner in 0.5 per cent concentration.

Solvent extracts from *mesua ferrea*, viz. petroleum ether, ether and chloroform extracts and oil obtained from the petroleum ether extract, and a pure crystalline compound from the plant were also tested. The petroleum ether, chloroform and ether extracts in 0.5 per cent concentration and the oil, inhibited the growth of *S. aureus*, *S. dysenteriae* and *M. phlei*.

4. *Stability of Panthenol*.—The preliminary experiments as given in the last report had shown that Mn^{++} and Fe^{++} ions had a deteriorating effect on panthenol in aqueous solution. Experiments were carried out to investigate in detail this effect and to find out if possible any relation between the concentration of Mn^{++} ions and the rate of decomposition of panthenol. It was found that the rate of decomposition of panthenol was proportional to the amount of Mn^{++} ions present. Attempts are being made to overcome this deteriorating effect of Mn^{++} ions on panthenol with the help of EDTA and other agents. The results of experiments so far carried out showed that EDTA had no stabilizing effect on the vitamin against the deteriorating action of Mn^{++} .

(9) Department of Virology

1. *Rabies A. Tissue Culture*.—Studies on rabies in this department have been directed to several aspects of the virus and its activities.

Both in applied research on different vaccines and their efficacy, as well as studies like purification and characterization of the virus, work is retarded by the necessity of mouse inoculations and titration in mice at stage, whether to estimate the virus or to estimate antibody by neutralization tests. Tissue culture seems to be a useful tool for shortening these procedures, but since no CPE is produced in Hamster Kidney Tissue Culture (HKTC) this virus, the difficulty of interpretation still remains. To try and study virus titres in tissue culture, it is proposed to make use of the interference phenomenon. It has been shown that rabies infections of the cell interfere with the uptake of other viruses, vaccinia, WN, Polio etc. It is proposed to adapt an enterovirus strain, non-pathogenic, to mice, to produce marked CP changes in HKTC and to study the interference produced by prior infection of the cultures by rabies virus. If replication of the rabies virus results in interference with the growth of enterovirus, absence of C.P.E. will indicate the presence of rabies. These fluids will be checked by mouse inoculation to confirm interpretation of results. If a consistent and reproducible interference effect is produced, it will enable us to make direct titrations of rabies virus, using the second virus as an indicator. Work on adaptation of ECHO 7 and ECHO 3 viruses to HKTC commenced early in the year, and is continuing.

Rabies B. Pathogenesis.—Rabies has a long "incubation" period in man and larger animals. Since it is known that the virus, on reaching the CNS, spreads throughout this system and produces symptoms within 48-72 hours, it appears that there must be some site where the virus remains latent during the long incubation period either until its titre is built up to a level capable of causing CNS infection or merely remains in latent form until it is reactivated by some stimulus which may be extrinsic or intrinsic. Such a latent period does not occur in small animals. It is considered worthwhile to try and produce such latency by artificial methods. As a preliminary experiment, mice are inoculated with virus and, after a short interval with antirabies serum. It has been shown that this method prolongs the incubation period in guineapigs. If a prolongation of incubation period is produced, further experiments, by direct as well as indirect methods, will be conducted to try and locate the site of the virus.

Batches of 10 mice are inoculated with approx. 10 LD₅₀ of CVS virus (mouse brain) by the subcutaneous route. Immediately (about 15-30 minutes later) the mice receive I.P. 1/2 ml. of antirabic serum, containing approx. 250 units per 1/2 ml. (1 unit neutralizes 30-300 LD₅₀ of virus. Controls are similarly inoculated with virus and with normal serum. Survivors in both groups will be observed upto 6-7 months. Three experiments have been done and the survivors are under observation.

2. *Vaccinia*.—Adaptation of vaccinia virus to Monkey Kidney Tissue Culture (MKTC) was successfully completed and the character and identity of this T.C. Virus were studied. Attempts at setting up a direct neutralization test (NT) in culture tubes containing liquid medium, were beset by the same

difficulty as has been reported in N.T. in eggs, i.e. there is a dissociation of virus and antibody, resulting in infection of the cells with only a slight delay as compared to control tubes without antiserum. In eggs this difficulty is overcome by estimating neutralizing titre of antibody as that which produces 50 per cent reduction in the pock count. A similar method in T.C. using 50 per cent reduction in plaques on agar overlay cultures has been described by Cutchin and Warren. This technique was adopted in concurrent studies with pock formation in eggs and a close parallelism was found in the resultant titres obtained. However, it was found that plaque formation by virus and inhibition by antiserum, in agar overlay cultures, was more consistent and reproducible than pock formation on the chorioallantoic membrane.

The paired sera of vaccinees were collected and tested for neutralizing antibodies by plaque inhibition, for C.F. antibodies and for HAI antibodies.

Analysis of the results indicates the following:—

(a) There is a broad correlative trend between prevaccination antibody titres (on HAI & NT) and the subsequent dermal response.

(b) The scatter in each group is too wide to admit of direct interpretation of immune status or prediction of response to vaccination.

(c) The degree of scatter is less when using a vaccine of high potency and the importance of this factor has been shown in the study.

(d) It appears possible to establish a marginal antibody titre (e.g. 1:40 HAI or 1:64 on N.T.) such that persons having antibody titres *above* the marginal level can be accepted as immune. This has considerable practical significance in small pox eradication campaign, since by a single screening test like HAI, it would be possible to separate out the cases in need of revaccination and surveillance. A larger series of cases needs to be studied from this point of view to establish acceptable "marginal" titres for this purpose.

3. *Influenza*.—During the period under review, 18 throat gargles, 13 throat swabs, and 7 paired blood samples were obtained from persons suffering from acute upper respiratory inflammation. Of the 6 samples analyzed so far, no isolation of influenza virus has been made. The remainder are under study.

4. *Measles*.—Studies on the efficacy of measles vaccine are being undertaken in collaboration with the D.G.H.S. (and I.C.M.R.) who will supply the vaccine and the Paediatric Centre of the J.J. Group of Hospitals, Bombay. As a preliminary to serological testing of blood from vaccinees cell cultures of human amnion and chick embryo are being prepared. These will be used for cultivation of standard measles virus strain obtained from U.S.S.R. for preparation of antigens.

5. *Stability of Oral Polio Vaccine (Sabin)*.—It was decided to test the stability of the concentrated vaccine bulk (received from U.S.S.R. in February 1966) by storing aliquots of 1 ml. at different temperature i.e. at room temperature, 4°C and -20°C. This study was started on 3rd September 1966. The aliquots are to be stored at room temperature for 7, 15, 22, 30, 45,

60 and 90 days ; at 4°C for 15, 30, 45, 60, 120, 150, and 180 days and at -20°C for 60, 120, 180, 240, 300 and 365 days. The work is still in progress. After storage at room temperature and at 40°C, the aliquots were transferred to a -20° C deep freeze cabinet and titrated in ten-fold dilutions in MKTC tubes. The end point results were read on the 7th day.

Results obtained indicate that on prolonged storage of vaccine even at -20° C there is a drop in the titre of the vaccine virus. Significant drop in the titre occurs after a storage period of 7 days at room temperature, 45 days at 4° C (refrigerator temperature) and approximately 2 years at -20°C.

Though the concentrated vaccine received by us was treated with $MgCl_2$ it was planned to study its stability after doing additional $MgCl_2$ treatment during dispensing also, instead of diluting in sterile Hanks containing 1 M $MgCl_2$. As pH of the fluid is known to play an important part, the diluted vaccine was kept in 2 flasks. In one flask, the pH was adjusted to 6.8 and in another to 7.2. Aliquots from each flask were stored at different temperature (i.e. room temperature (RT) ; 4°C and -20°C.). This study was started on 2nd November, 1966. The aliquots are to be stored and tested at refrigerated temp. for 4, 7, 10, 15, 20, 25, 30, 45 and 60 days, at 4°C for 7, 15, 30, 45, 60, 75, 90, 120 and 150 and 160 days and at -20°C every month for 2 years. After storage, the aliquots were transferred to -20°C deep-freeze and titrated as stated before in MKTC tubes.

The results obtained so far are summarized below :—

Studies on Stability of Stabilized Oral Polio Vaccine (Sabin)

			TCID /ml.	Drop in lgs
Original titre of the vaccine (diluted in Hanks 1M $MgCl_2$ —642).			4.00	..
Vaccine stored at 4°C for	7 days pH 6.8	..	4.00	..
	15 days pH 6.8	..	3.66	0.34
	15 days pH 7.2	..	4.00	0.00
	30 days pH 6.8	..	4.00	0.00
	30 days pH 6.8	..	4.00	+0.66
	45 days pH 6.8	..	4.00	0.00
Vaccine stored at R. T. for	4 days pH 6.8	..	3.50	0.50
	7 days pH 6.8	..	3.80	0.20
	7 days pH 7.2	..	3.50	0.50
	11 days pH 6.8	..	3.50	0.50
	15 days pH 6.8	..	3.66	0.34
	15 days pH 7.2	..	3.50	0.50
	20 days pH 6.8	..	3.00	1.00
	30 days pH 7.2	..	2.33	1.67
	45 days pH 6.8	..	3.00	1.00
Vaccine stored at -20° C for	30 days pH 6.8	..	4.66	+0.66
	30 days pH 7.2	..	4.33	+0.33

From the above result it is clear that the vaccine retains its original titre 4°C upto at least 45 days but at room temperature, even a storage of 20 shows a fall of one log i.e. 90 per cent of the virus activity.

There does not seem to be much difference in the vaccine stability when its pH was kept at 6.8 and at 7.2.

The work is still in progress.

6. *Preliminary experiments on storage of Monkey Kidney Cell Suspensions at 4°C .*—A few experiments of storage of monkey kidney cell suspensions were carried out with a view to determine as to how long such cells remain viable when stored at refrigerator temperature.

This work was started in the middle of June 1966 and so far kidney cell suspensions of six monkeys have been employed for this study.

The diluted cell suspension (with a cell count of 0.35 million cells/ml. suspended in M Hanks with 5 per cent horse serum) was kept in aliquots of 20 ml. amount each and stored in the frigidaire at $0^{\circ}\text{--}4^{\circ}\text{C}$.

After storage for 4, 7, 10, 14 and 30 days respectively, the suspension was seeded in tissue culture tubes in the following way :—

(a) The suspension was seeded in tissue culture tubes (0.5 ml/tube) as such, i.e. without centrifugation.

(b) The suspension was centrifuged at 7°C at 600 rpm. for 15 minutes, the supernatant fluid discarded and the cell deposit resuspended in fresh M Hanks (containing 4 per cent horse serum). This was seeded in tissue culture tubes. (0.5 ml/tube).

The tubes were incubated at 37°C and checked every day for cell growth. The medium was changed when the pH became acidic (varying from 4th to 7th day after seeding).

Results.—The growth of cells in almost all the batches was found to be rapid and satisfactory. The cells stuck to the glass surface and few even multiplied within the first 24 hours, showing "island formation."

From the preliminary experiments carried out so far, it is evident that the cells remain viable after cold storage for 7 days. The cell growth is quicker and superior in quality when cell suspensions are seeded as such (i.e. without centrifugation) than when seeded after centrifugation and resuspension.

When seeded as such, the cells form a complete monolayer cell sheath even after cold storage for 14 days in all batches and after storage for even 30 days in two batches. However, after cold storage of 14 days, when the cells are centrifuged and then seeded, they do not grow or grow rather poorly.

The tube seeded after 4, 7 and 14 days of cold storage are taken up for susceptibility studies for the three types of poliovirus and ECHO virus types 9, 10 and 14 (selected arbitrarily). These M. K. cultures were found to be susceptible to these viruses, though quantitative studies have not yet been done.

7. *Study of Non-specific Inhibitors in Normal Animal Sera against viruses.*—Sera from normal animals namely cattle, monkeys, horses, sheep dogs have been collected with a view to study the presence of inhibitors against polio virus types I, II and III the virulent as well as attenuated strains of each type.

The results of 25 cattle sera are already reported in the last report.

The results of 22 monkey sera are recorded as under :—

- (a) In general, more inhibitors were detected at 34° C than at 37° C.
- (b) 18/22 (81·27 per cent) sera showed the presence of inhibitors against one virus type or another.
- (c) Comparatively less inhibitors were present in sera against Type II Polio virus than against the other two types.
- (d) As in cattle sera, no appreciable difference was observed between the virulent and the attenuated strains as far as the presence of inhibitors is concerned.
- (e) 4/22 sera did not have any inhibitor against all six virus types.

Analysing the results of 24 sheep sera the following observations are recorded :—

- (a) 21/24 sera, i.e. 87·5 per cent showed the presence of an inhibitory substance against one polio virus type or the other.
- (b) It is of importance to note that three sheep sera contain inhibitors against all six virus types.
- (c) More inhibitors were detected at 34° C than at 37° C.

Analysing the results of 15 dog sera tested—

- (a) 7/15 sera, i.e. 49 per cent showed the presence of an inhibitor against one polio virus type or the other.
- (b) None of the sera showed the presence of inhibitor against all virus types.
- (c) It was observed that this inhibitory substance was present more against type I attenuated polio virus than against any other types, at both 34° C as well as at 37° C.
- (d) In general, more inhibitors were present at 34° C than at 37° C.

8. *Study of Extraneous Agents Present in Normal Monkey Kidney Tissue Culture (MKTC).*—During this study a total of 75 MKTC samples were tested for extraneous agents, by in-vitro and in-vivo methods (as described in the previous report). The extraneous agents encountered during this study were

the Foamy agents and the Haemadsorption (HA) agents. The Foamy agents were encountered in 100 per cent of the cultures, whereas HA viruses were encountered in 10·5 per cent of the cultures. None of the other extraneous agents namely B, LCM, Measles, Coxsackie and SV 40 viruses was encountered in the kidney cultures studies.

9. *Intratype Differentiation of Polio virus Strains.*—The present work relates to the characterisation of 160 strains of polio virus type-1. Two genetic marker tests, viz. 'd' marker and antigenic marker have been used for the determination of the genetic characters of the unknown strains.

Polio virus strains were derived from :—

- (i) stools of recognized cases of paralytic poliomyelitis prior to and after the administration of Live Oral Polio Vaccine,
- (ii) healthy contacts of paralytic cases,
- (iii) healthy carriers who were not in contact with known clinical cases and
- (iv) vaccinees who had received live oral polio vaccine.

All tests were performed in primary rhesus monkey kidney tissue cultures by plaque method.

Results.—(i) Of the 22 strains isolated from typical paralytic cases prior to the introduction of live oral polio vaccine, 20 had d+ character and 2 had d± character. The antigenic marker results indicated that 14 of these strains are heterologous, 4 are of intermediate and 4 show homologous antigenic character.

(ii) Of the 15 strains isolated from typical paralytic cases during early period of vaccine campaign, 13 had d+ character and 2 had d± character. All of 12 isolated from paralytic patients during early period of vaccine campaign show distinct heterologous antigenic character.

(iii) Of the 82 strains isolated from clinical cases of paralytic poliomyelitis after considerable portion of the susceptible population was immunized with live oral polio virus vaccine, 81 had d+ character, and 1 had d± character. The antigenic marker results indicated that 33 isolates are antigenically related to LSc., 2 ab; 19 strains are of intermediate antigenicity whereas 30 strains are distinctly unrelated to the vaccine strain.

(iv) Of the 13 strains isolated from the contacts of clinical cases, 6 had d+ character, 5 had d± character and 2 had d — character. 10 of the 13 strains appear to be heterologous, 2 homologous and 1 intermediate.

(v) All the 9 strains isolated from healthy carriers of no known history of contact with clinical cases had d+ character. The antigenic marker results indicated that 5 strains are homologous and 4 appear heterologous.

(vi) Of the 19 strains isolated from the vaccinees who had been administered live oral polio vaccine 4 had d character, 2 had d $\pm$ character and 13 had d—character. 11 of the 19 strains are antigenically related to vaccine strain, 1 intermediate and the rest 7 strains were heterologous to the vaccine strain.

It is of interest to note that since the oral polio vaccine has been used on a fairly large scale in the community, a sizable number of strains isolated from clinical cases of polio myelitis have been found to be antigenically related to the vaccine strain.

10. *Studies on genetic variants amongst Polio viruses.*—One hundred and fifty-two strains of polio virus type—1 isolated from :—

(i) Clinical cases of poliomyelitis before the introduction of live oral polio vaccine in India26 strains.

(ii) Clinical cases of paralytic poliomyelitis since the introduction of oral vaccine (But not from paralytic cases in the vaccinated children)..... 79 strains.

(iii) Clinical cases of paralytic poliomyelitis who had taken polio vaccine before the onset of illness.....4 strains.

(iv) Apparently healthy children who were in close contact with known patients of paralytic poliomyelitis.....13 strains

(v) Apparently healthy individuals with no history of contact with clinical cases of poliomyelitis.....9 strains

(vi) The children who were administered live oral polio vaccine..... 21 strains.

were selected for this study. All were examined for two genetic markers rct/40° C (ability to grow at 40°C) and 'A' marker (Aluminium marker) a physical property wherein is demonstrated the specific protection afforded by Al⁺⁺⁺ salt to the attenuated strains towards the thermal inactivation at 50° C for minutes.

These markers were determined on the strains passaged once in monkey kidney tissue culture. Stationary MKTC tubes were used and points of viral infectivity were calculated as 50% per cent cytopathic doses 0.1 ml. of virus suspension.

Results.—(i) Of the 26 strains isolated from clinical cases of prior to the introduction of oral live polio vaccine in the community, had rct/40°C+character and 8 had rct/40° C—character. The results of 'A' marker are, 3 had A $\pm$ character, while the rest 23 strains had A—character.

(ii) Of the 79 strains isolated from clinical cases since the introduction oral live polio vaccine 17 had rct/40° C+character, 4 had rct/40°C $\pm$ character, and 58 had rct/40°C—character. The results of 'A' marker are, 78 strains had A—character, while the remaining 1 strain showed A $\pm$ character.

(iii) rct/40°C marker and 'A' marker on the 3 strains isolated from clinical paralytic patients, who had been administered live oral polio vaccine and one strain isolated from a paralytic patient who had received salk vaccine before the onset of illness, indicated they were all negative in character.

(iv) Of the 13 strains isolated from the contacts of clinical cases of Poliomyelitis, 7 had rct/40°C+character and 6 had rct/40°C—character, while all strains showed A—character.

(v) Of the 9 strains isolated from apparently healthy individuals, 5 had rct/40°C+character, 2 had rct/40°C ± character and 2 had rct/40°C—character. The results of 'A' marker test showed that 8 had A — character, while 1 had A ± character.

(vi) Of the 21 strains isolated from the vaccinees (administered oral live polio vaccine), 19 had rct /40°C—character and 2 had rct/40°C+character. The results of 'A' marker test showed that 20 were A—character, while 1 had A ± character.

The rct/40° C marker indicates the presence of a wide variety of genetic characters among the groups of poliovirus isolates in the community. It can be concluded the 'A' marker test can be more useful to determine the progeny of vaccine virus than the wild strains.

11. *Studies on Nagpur Encephalitis*.—Material was collected by an epidemiological team from the V.R.C., Poona, at Pauni, a village near Nagpur, where encephalitis cases were reported. The specimens were sent to Haffkine Institute for study of possible enterovirus aetiology. 68 samples were received and were tested in MKTC and in infant mice.

The Results obtained so far are as follows:—

		<i>identity</i>	
No. of samples received	68	1. Polio II ...	5
		2. Cox. B1 ...	1
No. of samples inoculated in T.C. ...	67	3. Cox. B2 ...	2
		4. Cox. B4 ...	2
No. + ve in T.C. ...	52	5. Cox A9 ...	2
		6. NP ...	28
No. inoculated in infant mice ...	67	7. To be established and identified ...	12
No. + ve in infant mice ...	14		
No. — ve in both T.C. & Mice ...	15		

PART III

ROUTINE ACTIVITIES

ROUTINE ACTIVITIES

(1) Analytical and control department

The department continued to work mainly in the two rooms allotted to which were found inadequate for the increasing activities of the. The inadequacy of space has proved a handicap in embarking on stage of our activity, viz. the standardization of diagnostic reagents and of raw materials and the department had to confine itself to the first viz. the standardisation of the finished products manufactured by the

Shri A. K. Patil, Shri F. L. Saldanha and Kum. M. Advani joined this ment as J. S. Os. from June 1966.

In addition to the work undertaken in the previous years, the department over the standardisation of 'Triple Antigen'. It also developed a gical assay method for the standardisation of 'Pemon' which is now followed as a routine. This completes the take-over of standardisation control of all the products so far manufactured by the different departments the Institute. The department also pointed out the drawbacks in the employed and made useful suggestions to the manufacturing departments to make the products more uniform in quality.

The following table gives the number of different tests carried out during the year :—

Name of the Test		Product			No. of samples tested
(1) Sterility	..	..	(i) Bact. Vaccines	..	12,725
			(ii) Antirabic Vaccine	..	10,964
			(iii) Parenteral Bulk Solns.	..	9,500
			(iv) Antitoxins, Toxoid	..	6,674
			(v) Miscellaneous	..	400
(2) Pyrogens	..	..	Parenteral Bulk Solns., Plasma and Plasma Fractions.		1,056
3) Chemical and physical Exams.	..	..	Parenteral Bulk Solns.	..	1,025
			Vitamin Preparations	..	311
			Sulpha Drugs	..	107
			Miscellaneous other drugs	..	38
(4) Toxicity Tests	..	..	Sera and Vaccines	..	895
(5) Absence of Inf. Virus	..	..	Antirabic Vaccine	..	384
(6) Potency	..	..	Antitoxins	..	85
			Antivenine	..	30
			Toxoids	..	21
			Triple Antigen	..	3
(7) Formaldehyde	..	..	In Vaccines and Sera	..	180
(8) Microbiological Assay	..	..	Pemcn	..	42
(9) Preservatives	..	..	In Vaccines, Antitoxins etc.	..	849
Total					45,289

(2) Department of Bacteriology

During the period under review, the production of El tor cholera vaccine was continued. No plague vaccine was produced as the department had sufficient stocks to meet very small demands received.

The Public Health Department decided to undertake the trials for determining the efficacy of three Types of cholera vaccines, viz. (1) Conventional cholera vaccine (2) El tor vaccine (3) mixed El tor-cholera vaccine. All the above types of vaccines were prepared and supplied in required quantities. In addition to the above 12,000 doses of cholera vaccine were supplied to the Public Health department in 50 dose containers for trial of jet gun inoculation at Pandharpur.

It is proposed to issue T. A. B. vaccine in 10 ml. and 5 ml. rubber capped vials in future and some vials have already been issued.

The quantity of vaccines produced and issued is given below.—

Vaccine			Quantity produced	Quantity issued
			ml.	ml.
Plague Vaccine	...	...	Nil	62,740
Cholera Vaccine (Conventional)	...	...	4,02,800	75,13,912
El Tor Vaccine	...	...	27 65,600	22,56,785
El Tor Cholera (mixed)	...	...	71,77,378	21,06,550
T. A. B. Vaccine	...	...	2,09,460	5,14,168

The vaccines were issued to States other than Maharashtra also.

MEDIA SECTION

The Media Section continued the manufacture of media stains and reagents required for diagnosis of diseases and for testing drugs.

The manufacture of parenteral solutions also continued in full swing.

In addition to the above the department provides general facilities for sterilisation, cleaning of glassware, etc. The ampoule section supplies cut and sterilised ampoules to Departments of Bacteriology, Immunology and Antirabic Department. The production figures of the different items are presented below:—

Production of Culture Media

1. Casein Hydrolysate Cholera Plague	...	
2. Meat Digest Agar (Typhoid)	...	818 litres.
3. Agar Medium for Cholera Research	...	15784·6 litres.
4. Sterility Medium Anaerobic	...	109764·6 bottles
Aerobic	...	101736 bottles.

Production of Parenteral Solutions

Product	Quantity in
1. Glucose Saline	57,796
2. 5 per cent Glucose Solution	56,938
3. Normal Saline	31,737
4. 25 per cent Glucose Solution	1,583
5. A. C. D. Solution	50,219
6. Hypertonic Saline	Nil
7. Distilled Water	12,377
8. Sodium Lactate	2,469

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

1. Peptone Water	219·5
2. Standard Broth	357·7
3. Sterility Media (Acid Broth)	89·95
4. Cooked meat media (Anaerobic broth)	143·05
5. Standard Agar	778
6. Acid Agar	771·5
7. Hartley's broth	Nil
8. Mac Conkey's Medium	3·5
9. Mac Conkey's Agar	129·00
10. Löffler's medium	25·00
11. Lowenstein and Jenson's medium	18·00
12. Subouroud Medium	28·00
13. Typhoid Agar	818·00
14. Cholera Agar	249·00
15. Glucose Broth	52·4
16. Sugars for Biochemical Tests:	
(a) Glucose	30·1
(b) Lactose	21·1
(c) Sucrose	26·2
(d) Mannite	30·1
(e) Maltose	2·3
(f) Arabinose	3·650
(g) Xylose	3·6
(h) Dulcitol	24·4

Product				Amount Manufactured in Litres
17.	B. S. A. Medium	...	...	Nil
18.	Taurocholate Medium	...	...	23·2
19.	Citrate Saline	...	...	10·00
20.	Dubo's medium	...	...	Nil
21.	Lab-Lemco broth	...	...	24·00
22.	Lab-Lemco N. broth	...	...	3·00
23.	Potato Agar	...	...	Nil
24.	D. E. C. Medium	...	...	43·00
25.	Glucose Phosphate	...	...	10·00
26.	Normal Saline	...	...	1,9620·00
27.	Selenite F. Medium	...	...	14·5
28.	Plague Agar	...	...	Nil
29.	17 per cent sodium citrate	...	...	Nil
30.	K. citrate	...	...	1·25
31.	Thioglycholate medium	...	...	114·00
1.	Benedict's Solution (Quantitative)	...	...	506·00
2.	Albert's Stain No. 1	...	...	11·00
3.	Albert's Stain No. 2	...	...	18·00
4.	J. S. B. Stain No. 1	...	...	15·00
5.	J. S. B. Stain No. 2	...	...	27·00
6.	Löffler's methylene blue	...	...	7·00
7.	Indole Reagent No. 1	...	...	Nil
8.	Indole Reagent No. 2	...	...	Nil
9.	Carbol Gention Violet	...	...	4·00
10.	Carbol Fuchsin Ziehl Neelsen's stain	...	...	78·00
11.	Gram's Stain No. 1	...	...	16·00
12.	Gram's Stain No. II	...	...	27·00
13.	Hayen's Fluid for R. B. C.	...	...	15·00
14.	Methylene Blue (Aqueous)	...	...	64·00
15.	Oxalic Acid Solution for W. B. C.	...	...	18·00
16.	Saffranine 0·5 per cent (Aqueous)	...	...	11·00
17.	Basic fuchsin	...	...	12·00
18.	Carbolic Acid 5 per cent	...	...	3·00

(3) Department of Blood Bank

Staff.—Dr. W. N. Gatlwar, M. M. S., Class II, was promoted as Senior Scientific Officer, Class I, in the Department of Clinical Pathology from 22nd October 1966.

Dr. Smt. D. Chengappa, M.M.S., Class I, has been appointed in his place, she being promoted from M.M.S., Class III, since 8th February 1967.

Dr. M. B. Kalloorkar, M. M. S. Class II, was transferred to J. J. Group of Hospitals from 4th January 1967.

Dr. N. G. Chanderkar, M. M. S., Class II, was appointed in his place.

Smt. N. J. Shah, Junior Scientific Officer, resigned from 20th April 1966.

Training.—Large number of medical and paramedical personnel is being deputed by the Director-General of Health Services and other governments and they are being provided training in this branch of medical laboratory technology. A practical course was arranged in collaboration with Atomic Energy Commission, in plasma processing and freeze-drying technology for the benefit of the staff of other Institutes, where the plasma drying equipment is being supplied by the Atomic Energy Establishment. Total of 22 trainees attended the course.

As the number of blood donors was going down since the easing of the national emergency, attempts to collect blood from voluntary sources are being undertaken. In this connection, Assistant Director had a meeting with the Registrar of Poona University and principals of colleges in Poona and the visits of the mobile blood collecting unit are being arranged to various colleges there. Similarly, the industrial establishments, like Kirloskar Oil Engines have also been contacted and visits for mobile units are being arranged.

A blood donation centre had also been arranged in the "March of Time" exhibition with arrangements to collect blood from the visitors to the stall on the spot. There was very good response from general public. Arrangements have been made to obtain outdated plasma from big blood banks like Calcutta, Delhi, Poona, for plasma fractionation. One batch has been received from the Calcutta Blood Bank already.

Production activities.—The routine activities of the department, viz., collection of blood, processing and lyophilisation of plasma; Plasma fractionation and preparation of blood group reagents was carried on as usual. It has been possible to meet the full demands for blood group reagents.

I. Plasma Production :—

1. No. of Blood Donors—

1. Voluntary	...	...	...	464
2. From Prisoners	...	...	...	4,466
3. On payment	...	...	...	3,198
4. From hospitals	...	...	...	1,698
Total ...				9,826

II. *Dried Plasma prepared—*

Haffkine Institute	...	...	...	513·25 litres
Defence	...	...	...	628·25 litres
Total				<u>1,141·50 litres</u>

III. *Dried plasma supplied—*

Defence supply	...	...	...	900·00 litres
Other parties	...	...	...	544·00 litres
Total				<u>1,444·00 litres</u>

IV. *Plasma Fractionation Unit—*

No. of batches of plasma taken for fractionation	...	35
Quantity of plasma processed	...	800·00 litres
Ampoules of Gamma Globulin produced	...	$\left. \begin{array}{l} 3057 \times 1 \text{ ml.} \\ 600 \times 2 \text{ ml.} \end{array} \right\}$
Ampoules of Gamma Globulin Supplied	...	$\left. \begin{array}{l} 2112 \times 1 \text{ ml.} \\ 506 \times 2 \text{ ml.} \end{array} \right\}$
Human Fibrinogen produced	...	$\left. \begin{array}{l} 93 \times 150 \text{ ml.} \\ 37 \times 10 \text{ ml.} \end{array} \right\}$
Human Fibrinogen supplied	..	$\left. \begin{array}{l} 67 \times 150 \text{ ml.} \\ 18 \times 10 \text{ ml.} \end{array} \right\}$
Bottles of Pasteurised Plasma Protein Solution produced.		20 × 250 ml.
Bottles of Pasteurised Plasma Protein Solution supplied (for clinical trial).		20 × 250 ml.
Bottles of Albumin produced	...	20 × 100 ml.
Bottles of Albumin supplied	...	20 × 100 ml.

IV. *Blood Group Reagent Production Blood Grouping Sera prepared:*

Anti-A	...	...	...	21,593 ml.
Anti-B	...	...	...	19,354 ml.
Anti-A+B	...	...	...	1,074 ml.
Coomb's Reagent	...	...	...	364 ml.
Bovine Albumin	...	...	...	1,055 ml.
Anti C+D	...	...	...	1,098 ml.
Total				44,538 ml.

Blood Grouping Sera supplied

Anti-A	...	...	...	19,164 ml.
Anti-B	...	...	...	19,403 ml.
Anti-A+B	...	...	...	631 ml.
Coomb's Reagent	...	...	...	367 ml.
Bovine Albumin	...	...	...	799 ml.
Anti-C+D	...	...	...	300 ml.
Total				40,664 ml.

Rh. Test done (All the voluntary blood donors were tested for Rh. and the results communicated to them) ... 304

V. No. of Antivenine ampoules lyophilised ... 51,261

(4) Department of Chemotherapy

The year under review has been one of all-round progress. The research projects on the synthesis and screening of antimicrobial agents, cytotoxic substances, synthetic diuretics, C.N.S. active substances etc. were pursued with zeal and in a number of them the results have been gratifying.

In the studies of cytotoxic substances, reports on 20 compounds out of the 23 sent for evaluation to National Cancer Chemotherapy Centre, Bethesda U. S. A., were received. Several of our compounds were found to possess significant activity in a number of test systems. Most of the compounds were of low order of toxicity. A couple of compounds have passed the sequential screening in Lymphoid Leukemia 1210 (mouse) (a test system which is considered to give greatest predictability for potential clinical use) and are being considered for further study by C.C.N.S.C.

In the project on synthesis of psychotropic substances, out of the 50 compounds screened by C.S.I.R. Unit, at Department of Pharmacology, Sheth G. S. Medical College, 13 passed primary screening and have been taken up for secondary screening. Two of these have shown promise of turning out as good tranquilizers.

Thirty-five compounds were synthesised and screened for diuretic action. Some of these have shown substantial activity in rats.

Post-graduate research to support the departmental activity registered a good progress. Four students worked for M.Sc. degree and seven for Doctorate degree. Three students submitted their thesis for M.Sc. and four for Ph.D. During the year two scholars from Government of India and one Senior Scientific Assistant from C.S.I.R. worked in the department. Seven research papers were sent for publication during the period.

The Drug Screening Unit tested a large number of compounds synthesised in the department against a wide range of pathogenic micro-organism, while pilot plant investigations were carried out for the study of Chlorpropamide (anti-diabetic drug) with the available chemicals.

Clinical products division continued to manufacture various vitamin tablets, sulpha-tablets and peomon (antiseptic) etc. The production has been maintained in spite of difficulties in getting raw materials and other supplies.

*Statement showing the products manufactured during the period
April 1966 to March 1967.*

Sr. No.	Name of Product	No. of Tablets manufactured
1	Vitamin AD Tablets (paediatric)	103,15,400
2	Vitamin BC Tablets	278,27,524
3	Vitamin B1 Tablets	25,67,906
4	Vitamin B2 Tablets	3,57,900
5	Vitamin C Tablets	73,41,050
6	Vitamin K tablets	5,26,790
7	Acid Nicotinic Tablets	11,38,656
8	Vitamin BC No. 3 Tablets	10,70,600
9	Multivitamin Tablets	309,61,298
10	Vitamin A forte Tablets	1,72,400
11	Vitamin AD Tablets (Coated)	17,15,146
12	Magnesium Trisilicate Tablets	11,21,600
13	Nicotinamide Tablets	40,800
14	Calcium with Vitamin D Tablets	22,42,740
15	Tolbutamide Tablets	9,41,240
16	Sulphathiazole Tablets	22,61,500
17	Sulphadiazine Tablets	49,50,500
18	Sulphaguanidine Tablets	81,89,500
19	Sulphadimidine Tablets	30,80,000
Total		10,68,22,550
20	Peomon	23,230 litres
21	Sulpha Cream	170 Pounds,

(5) Department of Clinical Pathology

GENERAL

The department continued to discharge its various routine and activities.

Second batch of 12 students was admitted for one-year course of Diploma in Medical Laboratory Technology recognised by the University of Bombay.

The research scholars and post-graduate students continued their during the period.

The number of tests performed is as follows:—

Test Performed	No. of paid tests	Free outside
Histopathological examination	417	4,086
Smear for T. B.		92
Culture for T. B.	70	47
Animal test for T.B.	34	17
Diff. Blood Count	66	446
Total W. B. C. Count	53	443
Haemoglobin	43	421
Blood culture for typhoid		1,299
Blood culture for other (routine)	8	32
Urine culture	93	144
Stool culture	5	10
C.S. F. culture	2	1
Miscellaneous	35	129
Total R. B. C. Count	37	419
Autovaccine	54	5
Widal Test (Free)		1,356
Leptospire Agg. Test (Free)		
Diphtheriae smear and culture (free)		240
Wassermann test	7	143
Kahn test	154	402
V. D. R. L. test	117	305

Test performed	No. of paid tests	Free tests outside
Pregnancy test (Qual.)	1,329	1,071
Pregnancy test (Quan.)	138	74
Stool exam. routine	31	221
Semen examination	10	3
Stool cholera	18	
Urine routine	73	357
Smear for Gonococci		
Rideal Walker Co-efficient	50	92
Blood for M. P.	9	1
Blood for Prothrombin time	10	17
Throat swab for culture	2	4
Bone marrow smear	1	
Other smear etc.		
Blood for filariasis	1	
Antibiotic Sensitivity	73	197
Frei's Test		
Tuberculin Test		
C. S. F. routine cell count	1	45
Ascitic and other fluids count		
Matoux test		272
Cold Agglutination test		
Casoni's test		
Tetanus Culture	1	7
Total No.	2,942	12,153

The following Diagnostic Reagents were produced during the year under review:—

Dead Emulsions	4,49,330 ml.
High-titre Sera	4,335 ml.
Kahn antigen	17,020 ml.
Wassermann Antigen	1,610 ml.
Casoni's Antigen	391 ml.
Cobra Venom Solution	1,088 ml.
R. V. Venom Solution	1,467 ml.
Biological Stains	9,00,000 ml.

Total ... 13,75,241 ml.

(6) Department of Entomology

Shri R. S. Prasad was awarded a Ph. D. for his thesis entitled "Some studies on Rat fleas and their bearing on the host specificity."

Kum. K. M. Karnikar submitted her thesis for a Ph. D. on "Studies of Muscid flies of Bombay" on 31st January 1967.

Shri Chaturvedi and Shri Vad continued their work for a Ph. D. degree. Shri T G. Tare continued his work for M. Sc. in this department.

Shri M. S. Rao was trained in this department for advanced training in medical Entomology and Rodent Control, as a short-term trainee for three months. Eight other students T.T.C. Course were trained in the department.

The Assistant Director attended the International Symposium on Venomous Animals at Sao Paulo in July and the Indian Rodent Symposium at Calcutta in December 1966. He also toured Marathwada for plague epidemiological studies and Delhi and Calcutta for Rodent Symposium work. He presided over the meetings of the Laboratory Animal Sub-committee of Indian Standards Institution at New Delhi and Bombay and participated in the quarterly meetings of Plague workers of four south Indian States as well as the rat control meetings of Bombay Corporation and Agriculture Department, Government of Maharashtra.

Routine.—Approximately 71 inquiries were attended to during the course of the year in connection with the identification of rats, insects, and snakes and also for their control. Specimens of insects of medical importance and slides were supplied to three institutions for educational purposes. About 12 bait boxes were supplied to Zilla Parishads and municipalities in Marathwada region.

Production and Supply.—The total quantity of snake venom extracted and supplied during the year was 155.36 and 189.77 gms. respectively. Snakes of different varieties purchased were 1467, while those received gratis were six only. Total number of scorpions purchased were 2395 and the amount of venom extracted was 1.51 gms. A total of 144 frogs were also purchased for feeding experiments.

(7) Department of Immunology

Routine.—During the year, all antisera were manufactured by enzyme refining method. With enzyme refined sera, the purity and quality of sera are better though there is a loss of about 30 percent in unitage. The serum reaction is also markedly less and as a matter of fact no complaints of serum reaction have been so far received by us since marketing of enzyme refined sera.

No new construction could be taken up at Pimpri due to non-availability of funds. Increase in production will not be possible unless construction of urgent requirements, such as quarantine stables, ordinary stables, Main Laboratory and residential quarters for the staff is taken up at Pimpri.

The triple antigen manufactured with a new composition was sent abroad to National Institutes of Health (Washington), Medical Research Council (England) and Central Research Institute (Kasauli) for checking the potency. The reports from all these laboratories showed that it possesses more than adequate potency as compared with the standard. The new triple vaccine has now been released for regular sale.

The demands for tetanus toxoid were on the increase. Hence the production of tetanus toxoid was stepped up.

Dr. G.D. Dimitrov, research worker under Indo-Bulgarian Exchange Programme, is working on the research project "Fractionation and Study of Biologically important factors in Russell's viper venom". Fractionation of R.V. Venom is now being carried out on a preparative scale. It is proposed to purify from the venom the enzyme phospholipase A and to standardise it, since this has some demand from research workers in this country and abroad.

Among the highlights of the achievements of the Immunology Department in research during the year may be mentioned (1) the unravelling of the exact role of iron in the control of toxin production by *C. diphtheriae* and (2) the discovery of the presence of a Bacteriocine in certain *Clostridia* which inhibit the growth of *Cl. tetani*. The full significance of the discovery of the bacteriocine is at present being worked out.

Production				Litres
1. Tetanus Toxin				
(a) Peptic digest medium	..	..	..	270
(b) Protein-free medium	..	..	..	1,200
2. Tetanus toxoid				
(a) Peptic digest medium	..	..	..	93
(b) Protein-free medium	..	..	..	1,020
3. Diphtheria Toxin				
(a) Papain digest medium	..	..	..	72
(b) Protein-free medium	..	..	..	166
4. Diphtheria toxoid				
(a) Papain digest medium	..	..	..	28
(b) Protein-free medium	..	..	..	166
5. Adsorbed Tetanus Toxoid	..	..	..	10
6. Gas Gangrene (Welchii) Toxin	..	..	..	165
7. Pertussis Vaccine	..	..	..	40
8. Triple Antigen	..	..	..	Bulk 80 (Amp. 15)
9. Tetanus Antisera	..	..	..	419 litres
10. Diphtheria antisera	..	..	..	29.5 "
11. Gas gangrene antisera	..	..	..	107.5 "
12. Antivenin	..	..	..	332.0 "

(8) Department of Nutrition and Biochemistry

General (Nutrition).—Dr. V.N. Jai, S.S.O.I. (Nutrition) returned in 1966 after completing a W.H.O. Fellowship in Advanced Nutrition Food science for 13 months at the London School of Hygiene and Medicine and at Ibadan, Nigeria. Dr. (Kum). K. Chatterjee who was as the M.O. in April 1966 left in March 1967. The services of Kum. M.S. Kotnis, Factory Canteen Adviser (MMS II) has been spared to the F.A.O. U.N. for a period of 18 months from February 1967 on an assignment up to 1968. She is now posted as a Consulting Nutrition Officer at the of Agriculture at Malawi, Africa. During her absence, Dr. (Kum.) Arora, Senior Lady Nutritionist, has been promoted to officiate as F.C.A. Three Lady Nutritionists—Smt. Gupte, Kum. Pai and Kum. Mhatre resigned and left. Shri P. Divakaran, Junior Research Fellow, obtained his M.Sc. degree in Biochemistry and has rejoined the Department as a Senior Research Fellow. Shri S. S. Samant has been taken up as a Junior Research Fellow. The Research Scheme on “Studies in Lipid Metabolism with reference to dietary fat” has been continued upto February 1968.

Shri V.P. Shinde, J.S.O., obtained his M.Sc., degree by Research. Shri Gopal Rao was transferred from Chemotherapy to the Nutrition Department as Senior Technical Assistant.

Routine Tests.—182 tests including 26 assays of vitamins were carried out on 50 samples of foodstuffs and other materials for purity and nutritive value.

Field Unit.—The training of various functionaries under the statewide Applied Nutrition Programme with UNICEF aid, which commenced last year, at the Public Health Institute, was resumed in November 1966.

The depleted technical staff of the field unit continued the educational and other activities as part of the wider public health nutritive work. The salient features of the activities are summarised below :—

(1) *Diet-cum-Nutrition Survey.*—A clinical nutrition survey of the class III and IV employees of the Institute was carried out, using the ‘Rapid’ schedule of Nutrition Assessment of the I. C. M. R. About 920 staff members were covered. A detailed statistical evaluation is in progress.

(2) *Hospital Diets.*—While the general management and the serving of diets to patients is satisfactory, some of the hospitals frequently experience difficulties of a practical nature, e.g. certain items in the diet scales, such as Baker’s Bread, is not available in small towns or in other places the rising prices posed problems of how to replace a conventional food with equally nutritious substitute. Such difficulties have been overcome from time to time by implementing suitable modifications in the statutory diet schedule after Government approval.

Recently, the question of studying afresh the existing practice of serving food and milk to child patients have been taken up, with a view to formulate a practical diet scale for this group of invariably malnourished young children admitted in pediatric wards.

Government has been once again urged to approve the previously formulated diet scales for the other vulnerable group of patients—the mothers, admitted to maternity hospitals/wards for whom there is no provision at present for any special dietetic treatment, such as an additional allowance for milk, fruits and other protective foods.

(3) *Social Dietetics*.—Study of dietary conditions in residential institutions, industrial and other canteens, catering to homogenous segments of society has been a useful activity in the sphere of practical dietetics.

(a) *Residential Institutions*.—During the year, suitable diet scales were recommended for :—

- (i) Prisoners in State Jails ;
- (ii) Beggar's Home for Females, Chembur, Bombay ;
- (iii) The Western Railway's Training School, Udaipur, Rajasthan ;
- (iv) Workshop for the Blind, Worli, Bombay.

(b) *Industrial Canteens*.— During the year, 19 industrial canteens were visited in Greater Bombay and Thana District to evaluate the nutritional merits of the meal/snacks supplied to the employees. Suitable recommendations and suggestions are made to individual establishments to improve the quality/quantity of the food served. Besides advice is also given to enhance the nutritive value of popular recipes by improving the cooking practices, introducing nutritious food combinations, maintenance of hygiene and periodical check up, food handling etc. Actual cooking demonstrations of suitable food combinations for introduction in daily meals and snacks were given at some local industrial canteens.

Introduction of the following protein-rich processed foods were recommended to the industrial canteens :—

- (a) Defatted groundnut cake flour ;
- (b) 'Paushtik atta' (the above, mixed with wheat flour) ;
- (c) Multipurpose food (the above, with besan, vitamins and mineral).

(c) *Square Meal Canteen*.—This is a Government canteen providing balanced midday meal to the ministerial and other employees of the Government Secretariat, Bombay, at a subsidised rate. Regular visits were paid to the canteen to ensure that the quality/quantity of the individual food items is kept up.

(d) *General (Sachivalaya) Canteen*.—This is another canteen for the same secretariat staff, wherein standardised snacks, as also beverages, are provided. During the year, 6 non-vegetarian snacks served at the canteen were standardised, bringing the total to 28. This work has regularised the system of issuing various raw foodstuffs required for the daily preparations of snacks.

Education and Publicity.—The brochure on Nutrition and Dietetics, first published in 1951 as 'Road to Good Nutrition', has been very well received. Subsequently, it was revised twice and translated into Hindi, Marathi and Gujarati.

It is a matter for gratification that the Ministry of Community Development and Co-operation, Government of India, have recently evinced special to translate it into all regional languages of the country. We have, taken this opportunity to revise carefully the original English text and enlarge wherever necessary. Additional descriptive notes on essential—their functions, daily requirements and important sources have been incorporated. The dietary allowances are all given conveniently in the system. Additional tables on food values and nutritional merits of food are introduced to serve as ready reference. The revised and enlarged edition, now in the press, is about to be published soon under a title 'Towards better Nutrition.'

Experimental Cookery.—While the information on the nutritive value individual foods as purchased is readily available, there is paucity of information on the caloric and nutrient content of food combinations as prepared, served and consumed by the different communities. The work reported last year continued and some egg preparations were standardised.

At the instance of the Wheat Associates, New Delhi, several recipes from Milo (American variety of jowar) were tried. Similarly at the instance of the Bombay Municipal Corporation, several recipes were worked out for attending their Well-Baby Clinic.

In assessing the quality of protein present in the various recipes, the new concept of Net Dietary Proteins vis-a-vis Total Calories (NDpCal. %) has been worked out for many preparations. The protein value expressed in this way, denotes the proportion of dietary calories supplied by protein, corrected for protein quality.

Leaf Proteins.—Acceptability tests were planned with a protein-rich (60 per cent) powder prepared from lucerne or alfalfa by the C. F. T. R. I., It was incorporated into the following commonly consumed chapati, coriander wadi, potato wada and sandwiches. It is found that addition of this substance is not acceptable as far as its taste, colour and is concerned.

Formulation of recipes from subsidiary foods.—In view of the shortage staple cereals—wheat and rice—the Department developed further the of incorporating sweet potato—a non-cereal starchy root vegetable—into commonly consumed food preparations such as *kheer* etc.

Cookery demonstrations.—In view of its utility and popularity, the of educating the 'grihinis' by the actual demonstrations of nutritious, food combinations from locally available foods, has been further during the year, to cover many more welfare centres.

With the rising spiral of food prices, even the so-called class finds it difficult to provide adequate and satisfactory diets to their Hence the activity has been extended to organised groups of the segment of society, to enlighten the women-folk in nutritious food and scientific cookery.

In all, 35 such demonstrations have been given, covering FIVE Welfare Centres spread all over the Greater Bombay and one at Ulhasnagar (District Thana).

Health Exhibitions.—During the period, Nutrition Sections were arranged in 6 exhibitions in Greater Bombay sponsored mostly by voluntary organisations. Depending upon the theme of the exhibition, suitable low-cost nutritious recipes, along with inexpensive foodstuffs such as ragi, the leafy greens, sprouted pulses, carrot, papaya etc. are invariably displayed.

Food Exhibitions-cum-Competitions.—Our effort to popularise nutritious recipes have evoked favourable response from several social women's welfare organisations. Some of them have been stimulated in recent months to plan nutritious food and cookery art competitions amongst their members, though their enthusiasm is rather reduced this year in view of soaring prices and unavailability of many of the food-items. The Lady Nutritionists of the Department have been frequently requested to serve on the panel of judges on such occasions.

Popular Talks with Food Display.—In response to requests from social welfare organisations, talks on subjects of topical interest are arranged, catering to the needs of a broad spectrum of health-conscious groups, such as students of social sciences, social workers, the middle-class intelligentsia pursuing various vocations etc. Presence of a large gathering of educated citizens on such occasions, eager to learn modern trends in practical nutrition, is fully utilised to demonstrate to them, two or more of the newer nutritious food combinations formulated in the Experimental Kitchen. Their acceptance of these 'unfamiliar' but palatable and well-prepared recipes with visual appeal provides the practical experience to us, so basic to the more essential work of standardizing the recipes—as narrated earlier and popularising them amongst an ever-widening circle of nutrition-conscious population.

TRAINING IN PRACTICAL APPLIED NUTRITION AND DIETETICS

1. *Applied Nutrition Programme.*—The ANP, which aims at helping rural people to produce and consume food of high nutritive value, is now in the second year of its inception in Maharashtra State.

The second course of training the different categories of functionaries, viz., instructors at Gram Sevak (and non-gram-sevak) Training Centres, Sanitary Inspectors, Presidents of Zilla Parishads and Chairmen of Panchayat Samitis and the instructional staff of S. E. O. S., C. E. O. S., etc. was at the Public Health Institute, Nagpur, on October 1966.

Tutorials and Practical Demonstrations were arranged not only at the Public Health Institute, Nagpur, but also at the Gram Sevak Training Centre, Amravati and Sindewahi for the benefit of the trainees.

A list of about 30 low-cost nutritious recipes demonstrated to the trainees in the previous year, was compiled and sent to the Principal of the same Institute for distribution to the trainees who attended the above programme.

Pediatric Nurses.—Lecture-cum-demonstrations were arranged for the nurses attending the post-certificate course in Pediatric Nursing in Bombay. The training programme for the medical interns and the para-medical staff at the Rural Health Units will be resumed as usual.

PARTICIPATION IN SEMINAR/PANEL DISCUSSIONS

(1) *International Rice Year 1966.*—Kum. Kotnis, F.C.A., presented a paper on "The Place of Rice in the Diet" at a Seminar held at Poona, in connection with the National Rice Week Celebrations, in December 1966.

(2) *Diabetic Association of India, Bombay.*—Dr. (Kum.) Arora participated in a panel discussion on "Diet and Drug in the Management of Diabetes", organised by the Association to celebrate its Annual Day in April 1966.

(3) *Consultative Service.*—The Department continued to render help in an advisory capacity to Y. W. C. A., S. N. D. T. University, K. E. M. Hospital, the Diabetic Association of India and the like. Technical advice continued to be given (by correspondence) to institutions and individuals regarding their queries on nutritional merits of foods, problems in practical dietetic etc.

General (Biochemistry).—The laboratory continued to give effective service to the hospitals and attended to some special work whenever necessary.

Shri A. R. Advaney was given extension of leave to complete the Medical Technologists' Course in U. S. A. Kum. Shantha Nair returned from Czechoslovakia after one year's advanced training at the Institute of Microbiology, Prague. Kum. Maya Advaney was transferred to the Quality Control Department after promotion. Kum. Phadnis who had passed the D.M.L.T. Course was appointed as Senior Technical Assistant. The Department has undertaken the additional work of training students for the D.M.L.T. Course. Eight students have opted for special training in Chemical Pathology. Special lectures and laboratory work is arranged for them.

Shri M. J. Modak, who completed the Maharashtra Government Fellowship, was awarded a Junior Research Fellowship (Diamond Jubilee).

Routine Tests

Blood	..	..	..	2,840
Urine and Stools	..	..	..	243
Body Fluids	..	..	..	99
Special Tests	..	..	..	710
Milk	..	..	..	5
				3,897

(9) Department of Pharmacology

General.—During the year under report, as in previous years, the Department continued its full activities in research, standardization of drugs, training of personnels and in post-graduate studies.

The Department had a distinguished guest worker, Dr. Vaughan Williams D. M. D.Sc., an eminent Pharmacologist of the Oxford University, who participated in the Cholera Research Project. He worked here from November 1966 to March 1967.

One of the contributions from this Department is in the field of Indigenous Drugs. There are a few instances in the history of Indian Medicine, where a drug has been passed through different stages of laboratory investigations, clinical trials and finally put into the market. The work on berberine, in alkaloid isolated here, from the Indian medicinal plant "*Berberis aristata*" in this respect is noteworthy. Berberine was first shown to be effective in experimental cholera of rabbits in 1963. Its study on toxicity has indicated that it would be safe when given therapeutically. A clinical trial of this drug carried out in Calcutta in co-operation with local hospitals for three successive years 1964 to 1966 show that berberine hydrochloride tablets are effective in clinical cholera and severe diarrhoea cases. Berberine has now been introduced into the market.

Dr. N. K. Dutta, D. Phil. (Oxon.), D. Sc. (Oxon.), F. N. I., Head of the Department, participated in the Eleventh Pacific Science Congress held in Tokyo in August 1966. He also visited important laboratories in U.S.S.R. and gave lectures under Indo-Soviet Scientific and Cultural Exchange Programme in November-December 1966.

Kum. S. V. Babrekar, M.Sc., is appointed as Research Assistant, Indian Council of Medical Research, Kum. Dr. S. E. Bhagwager, B.Sc., B.V.Sc., joined as Unicef Research Fellow, Kum. Usha Kashyap, M.Sc., resigned and left for U.S.A., Dr. Kum. Amita Mukherjee, M.B.B.S., Research Officer, Indian Council of Medical Research, also resigned and left for U. K. Shri A. D. Nadkarni, B.Sc., is appointed as Junior Scientific Officer (Biochemistry) and Kum. S. G. Kondeji, B.Sc., as Junior Scientific Officer (Chemistry).

Amongst those people, who were trained at this Department, mention may be made of Dr. N. M. Sathe, M.B.B.S., Civil Assistant Surgeon, Institute of Preventive Medicine, Hyderabad (Biological Standardization), Shri George Verghese, Drug Control Administration, Kerala (Biological Standardization), Shri V. J. Thomas, B.Sc., B. Pharm., Bacteriologist, Drug Control Administration, Kerala State (Chemical and Microbiological), Shri Sukchitta Taitilananda, B.Sc. (Pharmacy), Food and Drugs Inspector, Ministry of Public Health, Thailand, underwent training here under the Colombo Plan Technical Training Scheme. Eight graduate students were trained under the Technician Training Course. Various aspects of the Drug Testing were studied by Kum. R. N. Mehta of Bharat Laboratories and Shri S. W. Desai (Private Student). Shri B. P. Pandit of Mercuric Pharmaceutical Industries, Baroda, is under training.

Analytical Services (Routine activities).—This department has specialised in the fields of analysis of biological and chemical products. The samples were received from various sources, namely, Drugs Control Administration, Government of Maharashtra, Department of Prohibition and Excise and Police, Government Hospitals of Maharashtra State, pharmaceutical firms etc. Variety of samples that were received belonged to pharmacopoeial and non-pharmacopoeial drugs, which required pharmacological, chemical, biochemical, pharmacognostic, microbiological and serological testing, depending the nature of the samples. Out of 3,009 samples that were tested, about 15 per cent were of non-standard quality. The percentage of non-standard drugs in 1965 was 14.2. Under the Drugs and Cosmetics Act, 1,573 samples examined out of which 8.6 per cent did not comply with the claims made. This figure is a distinct improvement over the quality of medicines examined during the previous year, in which about 12 per cent of the samples were of standard quality. Standardization of drugs on behalf of commercial continued to be popular. During this year as many as 488 samples examined on behalf of commercial organisations.

This Department collaborated with Central Drugs Laboratory, Calcutta, for establishing National Reference Standards for streptomycin and dihydrostreptomycin.

Biological Section.—Considerable headway has been made in giving a hand to the Insulin manufacturers of Maharashtra. Fifty-one samples Insulin were tested biologically which involved the use of a very large number mice. This has saved considerable foreign exchange being drained out of the country. There has been 180 per cent increase in testing capacity of the insulin of preparations as compared to last year.

A very large number of samples of antibiotics such as Chloramphenicol, streptomycin, tetracyclines, etc., were tested for undue toxicity. Out of two hundred and ninety samples tested, four failed to comply with the pharmacopoeial specifications for this test. Out of fifty-one samples of crude liver extract that were examined, 8 preparations did not comply with the one or the other official requirements. Out of 136 samples referred for pyrogens, sixteen samples failed to comply with the test. LD_{50} or acute toxicity was carried out in four samples. These were preparations containing phenyl butazones and amidopyrines. Forty-eight samples of adrenaline and four samples non-adrenaline were examined and only one sample of non-adrenaline was found to be less than the labelled amount. Six preparations of pituitary extracts were sent to us for standardisation. In these were included bulk samples before marketing.

Biochemical and Microbiological Section.—The samples analysed by this section consisted of vitamin formulations, antibiotics, and their preparations enzyme preparations (containing papain, pepsin, diastase etc.), antitoxins, vaccines, sera etc. One hundred and thirtythree samples of antibiotics and their related preparations were tested. Only a few samples of ointments of pericillin, and chloramphenicol with benzocain ear drops failed to comply with the standards for antibiotic activity. The percentage of such failures

was 3. Some of the samples of tetracycline hydrochloride were found to have changed colour and consistency. In one chloramphenicol sample the nett content per capsule was found to be less. The number of samples of vitamin preparations received for testing was much less than during the previous years. There seems to be considerable scope for work in studies on stabilisation of individual ingredients in multi-vitamine oral preparations. In 20 out of 180 such preparations, one or other important ingredient did not conform with the claim. A few samples of multi-vitamin preparations were found to be fortified with a very high percentage of extra vitamins. A number of injections of thiamine hydrochloride were found to be turbid and contained suspended particles. Eleven per cent of Vitamin B12 preparations failed to conform to the labelled amount. Diastase is now used in a number of multi-enzyme preparations. But in most cases, the diastatic activity was not correctly mentioned. None of the samples analysed for diastatic activity was found to have sustained the claim. Twenty samples of anti-toxins and 18 samples of vaccines were analysed. All the anti-toxin samples conformed to the claim.

Chemical Section.—During the year under review this section dealt with 1,458 samples. The samples consisted of simple and compound pharmacopoeial and non-pharmacopoeial preparations comprising of various categories which included fine chemicals, analgesics, anti-pyretics, narcotics, tranquilizers, antidiabetics, haemostatics, respiratory stimulants, antihistaminics, surgical dressings, insecticides, disinfectants etc. Unlike the previous years a greater number of fine chemicals like hexamine, sodium salicylate, borneol thymol, salts of sodium p tassium and iron etc. were referred to for testing. Many samples of the nature of anti-septics, insecticides and disinfectants were not properly labelled. R. W. coefficient was not indicated. Out of 130 such samples examined, only 73 confirmed to the claim made. Other such preparations stated to be "cresolis saponatus" did not pass the I. P. test for solubility. Seventy-six samples of ointments including pain balms and antiseptic preparations were tested. Thirty of them were found not to be of good quality. Some of these were unlabelled, some were claimed as Ayurvedic preparations. These pain balms were usually mixtures of essential oils having similar chemical properties. The presence of thymol, menthol, and methyl salicylate were detected in some using the technique of thin layer chromatography. In some of the unlabelled samples claimed to be useful for cold, eucalyptus oil was detected by the above technique. A few ointments labelled as Ayurvedic and claimed to be effective for itching, eczema etc. contained boric acid, salicylic acid, sulphur and sulphonamides. In a number of samples (12 out of 38) of gripe water fungus like growth and turbidity were observed. A few samples of cosmetic preparations were tested. These were not properly labelled. Out of the 10 samples of "Face powders", seven were found to conform to the claim. Six hundred and four samples were examined on behalf of Prohibition and Excise and Police Department. These included mainly spirituous medicinal preparations, cologne water, and samples labelled as "Lal Pari". Amongst them as many as 283 preparations were declared as of non-standard quality. Thirty-four "red" capsules were found to contain a sedative secobarbital sodium.

(10) Department of Virology

Routine.—Diagnostic studies were carried out on material referred from cases suspected of rabies (animal and human) and also from patients of miscellaneous clinical conditions suspected of viral aetiology. These are given in the tables below:—

Diagnostic studies for Rabies

Specimen received*	No. of Samples	No. +ve by smear	No. +ve by mice inoculation	No. —tive.
Dog Brain ..	12	4	6	6
Human Brain ..	2	Nil	Nil	2
Other animals (Bullock, cat, monkey, fox horses).	5	Nil	1 (Bullock)	4
Total ..	19	4	7	12

*No salivary glands were received for diagnosis.

Production—

Antirabic Vaccine	..	4,488,984	ml.
Canine Vaccine	..	22,086	ml.
Polio Vaccine	..	709,169	doses.

Materials from clinical cases referred to Haffkine Institute for diagnosis from various sources

Name of Institution	No. of samples received	No. of serum samples	No. inoculated for virus isolation in TC. Mice	Pending inoculation	Positive		Negative both in TC/Mice	Identity				
					T.C.	Mice		Polio	Non-polio	Cox	Awaiting results	
R. M. C.	..	14	..	12	2	6	0	6	..	3	..	1
Private	..	40	12	14	14	6	0	6	..	5	..	1
K. E. M.	..	6	2	1	3	1	0	..	..	1	..	..
L. T. M. G.	..	24	8	12	4	6	0	6	1 PI	3	..	..
B.J.W.	..	14	3	10	1	4	0	6	..	4	..	2
Total	..	98	25	49	24	23	0	24	1 PI	16	..	4

(11) Animal House

The Animal House work was in full swing during the year under report. All the requirements of the department were met. There was shortage Guinea pigs in the last quarter of the year and 245 Guinea pigs were procured from other laboratories. The breeding again improved from the month March 1967.

Training.—Students of the Technicians' Training Course and D.M.L.T. Course were trained.

During the year following persons were trained in the Animal House—all the aspects of the Breeding and Management.

1. Shri M. L. Sultan ... Regional Research Laboratories, Jammu, Kashmir.
2. Shri K. R. Bhardwaj ... Central Research Institute, Kasauli.
3. Shri M. N. Joshi ... Chougule and Company, Bombay.

Following figures will show the comparative production with the year 1965-66

Serial No.	Species	1966-67	1965-66
1 White Mice		1,77,130	1,62,840
2 Albino Rats		10,604	11,390
3 Rabbits		2,876	3,544
4 Guinea pigs		3,121	4,391
5 Hamsters		1,259	1,189
6 Cotton Rats		141	71

Besides these animals following species of animals were maintained in the Animal House and Stables of the Institute and issued to departments:—

- | | | | | |
|------------|-----|-----|-------|--------------------------------|
| 1. Sheep | ... | ... | 3,167 | |
| 2. Monkeys | ... | ... | 94 | } Maintained average per month |
| 3. Cats | ... | ... | 10 | |
| 4. Horses | ... | ... | 250 | } Including Pimpri Study Farm. |
| 5. Poultry | ... | ... | 120 | |

(12) Library Section

The Library of this Institute caters to the needs of our research and research workers of the other scientific and technical institutions. It also participates in the Inter-Library loan system which is in vogue in other neighbouring libraries.

The following statistics speak of the work attended to by the library during the year under report:—

A. Readers—

(1) No. of visitors in the library for reference only	...	867
---	-----	-----

B. Loan of books and periodicals—

(1) No. of books and periodicals issued to our staff	...	340
(2) No. of books and periodicals issued under inter-library loan to other libraries.		490
(3) No. of books and periodicals obtained for our staff from other libraries.		59

C. Additions of Library material during the year—

(1) Total number of books (Accn. Nos. 10584 to 10773)	...	190
(2) Total number of bound volumes of periodicals (Accn. Nos. 8615-9404).		790
(3) Total number of current periodicals subscribed	...	125
(4) Total number of current periodicals in exchange	...	144
(5) Total number of current periodicals as gratis	...	21
(6) Total number of periodicals in the current list	...	290
(7) Total number of reports of scientific institutions received in the library.		58

(A) LIST OF PAPERS PUBLISHED

1966-67

Department of Analytical and Control

1. Saldanha, F. L. Cholera El Tor in Maharashtra : *Indian Journal of Med. Res.* Vol. LIV, No. 12, 1966, pp. 1097-1101.

Department of Bacteriology

2. Nimbkar Y. S. and Karbhari R. S. Biological Assay of Cholera Vaccine : *Indian Journal of Pathology and Bacteriology* Vol. IX, No. 2, April 1966, pp. 139-150.
3. Deshmukh, Kalindi. Acid Soluble Nucleotides of *Vibrio cholerae* (Inaba) : *Indian Journal of Microbiology*, Vol. VI. Nos. 1 and 2, January-June 1966, pp. 47-52.
4. Gadgil, M. D., Nimbkar, Y. S. and Jhala, H. I., Morphological Variations of *P. pestis* in relation to requirement of Ca^{++} and Mg^{++} : *Indian Journal of Pathology and Bacteriology*, Vol. IX, No. 4, October 1966, pp. 323-330.

Department of Blood Bank

5. Parikh, N. P. and Kuikarni, K. V. Bacterial Contamination of Banked Blood and Evaluation of use of Sulpha Drugs in its prevention : *Indian Journal of Pathology and Bacteriology*, Vol. X, No. 1, January 1967, pp. 56-61.

Department of Chemotherapy

6. Umerji, G. M. and Bellare, R. A., Hair Manganese Levels in Normal Subjects : *Indian Journal of Expt. Biol.*, 4(4) : pp. 212-214, 1966.

7. Patel (Smt.) M. R., Bellare, R. A. and Deliwala, C. V. Antitubercular action of Vakeri (*Caesalpinia digyna Rottl.*) *Indian Journal of Expt. Biol.*, 4(4) : 214-215, 1966.

8. Shah, L. P., Vasavda, M. P., Bagadia, V. N. Sheth, U. K. and Deliwala, C. V. Further experiments with a stable fraction of Harik-Papspalum Scrobiculatum. (Symposium on C. N. S. Drugs, 1966).

Department of Clinical Pathology

9. Sant, M. V. and Limaye, S. S. Tuberculous Endometritis (A histological Study of 301 Cases) : *The Journal of Obstetrics and Gynaecology of India* Vol. XVI, No. 2, April 1966, pp. 205-212.

10. Vernekar, Sharad, D., and Sant, M. V. The 'in vivo' and 'in vitro' Studies of Antibody Production : *Indian Journal of Pathology and Bacteriology*, Vol. IX, No. 3, July 1966, pp. 246-257.

11. Iyer, Laxmi, S. and Sant, M. V. Studies on Antibigram pattern, phage typing and Serological Classification of Pathogenic Staphylococci : *Indian Journal of Medical Sciences* Vol. 20, Spl. No. 11, Nov. 1966, pp. 770-779.

12. Ghag Nalini K. and Sant, M. V. Rapid Laboratory Diagnosis of Diphtheria Fluorescent Antibody Technic (Part I) : *Indian Journal of Medical Sciences* Vol. 20, No. 12, Dec. 1966, pp. 907-913.

13. Ghag Nalini K. and Sant, M. V. Rapid Laboratory Diagnosis of Diphtheria Fluorescent Antibody Technic (Part II) : *Indian Journal of Medical Sciences* Vol. 20, No. 12, Dec. 1966, pp. 914-919.

14. Modi, B. G., Das K. K. and Iyer, L. S., Serum agglutinin following a T. A. B. Inoculation : *Indian Journal of Pathology and Bacteriology*, Vol. 10, No. 1, January 1967, pp. 50-54.

15. Belgaumkar, P. H. and Sant, M. V., Studies on Antibiotic Sensitivity, Bacteriophage types of *S. typhi* and *S. paratyphi*. A Strains and Vi agglutination contents of enteric fever cases : *Indian Journal of Medical Research*, Vol. IV, No. 3, March 1967, pp. 199-204.

Department of Entomology

16. Deoras P. J., Tolerance of an Anticoagulant by *Rattus rattus* : *Current Science*, Vol. 35, No. 16, August 20, 1966, pp. 415-416.

17. Deoras P. J., Studies on Bombay Rats : *Current Science*, Vol. 35, No. 18, September 20, 1966, pp. 465-466.

Department of Immunology

18. Bundeally Amina E. and Rao S. Srinivasa Amino Acid and Vitamin requirements of *Bordetella pertussis*. *Indian Journal of Expt. Biology* Vol. 4, No. 2, 1966, pp. 124-125.

19. Sardesai S. K. and Rao S. S. Role of iron in the regulation of toxin production by *Corynebacterium diphtheriae* : *Indian Journal of Expt. Biology* Vol. 4, No. 4, 1966, pp. 209-211.

20. Acharya U. S. V. Buduk, D. P. and Rao, S. S. Changes in Horse Serum Proteins and Antibody Proteins after Hyperimmunization and repeated bleedings : *Indian Journal of Biochemistry*, Vol. 3, No. 2, 1966, pp. 128-130.

21. Mathew, Thankam, Cultivation and Immunological Studies of the Pox Group of Viruses : I. Propagation of vaccinia virus in Hamster Kidney Cell Culture : *Indian Journal of Pathology and Bacteriology*, Vol. IX, No. 3, July 1966, pp. 207-213.

22. Mathew Thankam, Cultivation and Immunological Studies on Fox Group of Viruses : Part II. Haemagglutination and Haemagglutination Inhibition Studies of Avian Pox Viruses by plate agglutination : *Indian Journal of Pathology and Bacteriology*, Vol. X, No. 1, January 1967, pp. 1-8.

23. Mathew Thankam, Virus Study of peck disease among buffaloes (Letter to Editor) : *Indian Journal of Pathology and Bacteriology*, Vol. X, No. 1, January 1967, pp. 101-102.

Department of Nutrition and Biochemistry

24. Nair, Shanthakumari, Modak, Mukund, J., and Venkataraman, A. Cellular Constituents of *Pasteurella pestis* : *Indian Journal of Biochemistry*, Vol. 3, No. 3, 1966, pp. 206-208.

25. Soman U. P. and Ambegaokar S. D. Effect of Commercial Sterilization in the nutritive value of buffalo milk-Part II : Amino Acid Composition and Protein Efficiency Ratio. *Journal of Nutrition and Dietetics* October 1966, Vol. 3, No. 4, pp. 117-120.

26. Arora, Shanti, Standardisation of some common food preparations : "*Madhumeha*", Vol. VII, No. 3, January 1967, pp. 143-146.

27. Modak, M.J. Divakaran, P. and Venkataraman, A. Blood Cholesterol in Donors. *Lancet*, 18th February 1967, No. 7486, Vol. I, pp. 388-389.

28. Shirde, V. P. and Ambegaonkar, S.D. Pantothenic Acid sparing action of Vitamin B-12 in Pantothenic Acid deficient rats ; *Indian Journal of Experimental Biology*, Vol. 5, No. 1, January 1967, pp. 42-49.

29. Jai, V.N., Nutritional Determinants in 'Heart' Diseases : *Bombay Civic Journal*, March 1967, pp. 1-4.

Department of Pharmacology

30. Lahiri, S.C., and Dutta, N.K. Berberine and Chloramphenicol in the treatment of Cholera and Severe Diarrhoea : *Journal of Indian Medical Association*, January 1, 1967, Vol. 48, No. 1, pp. 1-11.

Department of Virology

31. Ver, Binie, A., Nanavati, A. N. D. and Jhala, H.I. Kinetic studies on growth of Rabies Virus in Tissues Culture. Part I : Cell Virus Interaction. *Indian Journal of Pathology and Bacteriology*, Vol. IX, No. 2, April 1966, pp. 101-111.

32. Ver, Binie, A., Nanavati, A.N.D., and Jhala, H.I. Kinetic Studies on growth of rabies virus in Tissue Culture, Part II : The Replication Cycle in Rabies Virus Interaction. *Indian Journal of Pathology and Bacteriology*, Vol. IX, No. 2, April 1966, pp. 112-122.

33. Mansharamani, H.J., Dandawate, C.N., and Krishnamurthy, B.G. Experimental Vaccine against Kyasanur Forest Disease (KFD) Virus from Tissue Culture Source : Part I. Some data on the preparation and antigenicity tests of vaccines. *Indian Journal of Pathology and Bacteriology*, Vol. X, No. 1, January 1967, pp. 9-24.

34. Mansharamani, H.J., and Dandawate, C.N. Experimental Vaccine against Kyasanur Forest Disease (KFD) Virus from Tissue Culture Source. Part II : Safety Testing of the vaccine in Cortisone Sensitized Swiss Albino mice. *Indian Journal of Pathology and Bacteriology*, Vol. X, No. 1 January 1967, pp. 25-32.

Polio Research Unit

35. Samuel, M.R. and Bhagwat, S.M. A Clinical Study of hundred cases of Poliomyelitis *Indian Journal of Med. Sciences*, Vol. 20, No. 11, November 1966, pp. 757-764.

36. Bhagwat, S.M. and Samuel, M.R., Oral Polio Vaccine (Sabin) Vaccine Associated Illnesses : *Indian Journal of Medical Sciences*, Vol. 20, No. 12, December 1966, pp. 903-906.

(B) LIST OF PAPER AWAITING PUBLICATION*Department of Bacteriology*

The role of Nutrients and Temperature in the growth of *P. pestis* for vaccine production (Part I) : Gadgil, M.D., Nimbar Y.S. and Jhala H.I. (Indian Journal of Pathology and Bacteriology). The role of Nutrients and Temperature in the growth of *P. pestis* for vaccine production (Part II) : Gadgil, M.D., Nimbkar, Y.S. and Jhala H.I. (Indian Journal of Pathology & Bacteriology). Temperature induced growth imbalance of *P. pestis* in relation to RNA, DNA and protein synthesis. Gadgil, M.D., Nimbkar, Y.S. and Jhala, H.I. (Indian Journal of Pathology and Bacteriology).

Department of Blood Bank

Blood, its components and their clinical uses : Part I. Kulkarni, K.V., (Maharashtra Medical Journal, Poona).

Blood components and their clinical uses : Part II. Kulkarni, K. V. and Karkhanis, C. J. (Maharashtra Medical Journal, Poona)

Department of Chemotherapy

3, 4-Di-substituted-5-Carboxy Methylmercapto-1,2,4(H)-thiazoles as possible antiviral agents. Shah, M.H. and Mhasalkar, M.Y., Varaya, N.A., Bellare, R.A., and Deliwala, C.V. (Indian Journal of Chemistry).

In-vitro of 1-Acyl-4-Alkyl (or Aryl)-Thiosemicarbazides 1-5-(Chlorosalicylidine) - 4-Alkyl 1 (or Aryl)-Thiosemicarbazones and some Hydrazones of 5-Chlorosalicylaldehyde against Pathogenic Bacteria including *M. tuberculosis* (H37Rv). Bhamaria, R.P., Bellare, R.A. and Deliwala, C.V. (Indian Journal of Chemistry).

Stability of Folic Acid in Presence of Thiamine Hydrochloride. Kalokhe, Vilasinee, P. and Colah, R.B.M. (Indian Journal of Pharmacy)

In vitro effect of isorazoline, Pyrazoline and Thiazole Derivatives against *M. tuberculosis* H 37 Rv and other bacteria : Patel, (Smt.) M.R., Bellare, R.A. and Deliwala, C.V. (Indian Journal of Microb.)

Synthesis of Potential Anticancer Agents derived from Benzaldehyde Nitrogen Mustard and Aminophenylthiazoles. Sabnis, S.S. (Indian Journal of Chemistry)

3,4, 5-Trimethoxyphenyl Amino-Alkyl-Ketones, Amino-Alcohol and Amide Derivatives as CNS Depressants. Patigara, R.B., and Deliwalla, C.V. (18th Ind. Pharmaceutical Congress, Bombay) Synthesis of Potential anti-cancer Agents. Dhaplapur, M.G. and Deliwala, C.V. (18th Ind. Pharm. Congress, Bombay).

Department of Entomology

Histology of the Neurosecretory system of Adult *Musca nebulosa* : Deoras, P. J. and Bhaskaran, G. (*The University Journal*) Studies on House Flies : Deoras, P.J. and Karnikar, Karuna, (*Current Science*)

Observations on the morphology of *Echis carinatus*: Deoras, P.J. and Vad, N.E. (*The University Journal*).

Feeding Mechanism of Indian Fleas *X. cheopis* (Roths) and *X. astia* (Roths) Deoras, P.J. and Prasad, R.S. (*Annals of Tropical Medicine and Parasitology*)

Department of Immunology

Fractionation of the Venom of *Vipera russelli* by gel Filtration. Part I : Distribution of some enzyme activities and the toxic factor during gel filtration.

Fractionation of the Venom of *Vipera russelli* by Gel Filtration. Part II- study of yellow and white venoms of *Vipera russelli* with special to the dermonecrotic and toxic actions. Dimitrov, G.D. and Kankon- R.C.

Department of Nutrition and Biochemistry

Cellular Constituents of the Salmonella. Nair, Shantakumari, Modak, Mukund, J. and Venkataraman, A. (*Indian Journal of Path. and Bact.*)

Serum Glycoprotein Components in Health. Mehta, M.G. and Venkata- A. (*Journal of Clinical Pathology*, London).

Nutrition Programme (A.N.P.) in Maharashtra : Jai, V.N. (*Civic Journal*).

Effect of Pantothenic Acid on Blood regeneration. Shinde, V. P. and S.D. (*Journal of Nutrition and Dietetics*).

A study of the Diet Pattern of the Sindhi Community in Bombay : Advaney, and Venkataraman, A. (with the statistical assistance of Rao, N.R.) (*of Nutrition and Dietetics*).

Department of Pharmacology

Cholera Field Vaccines and Rabbit Test—Laboratory Assay of Field Trial Cholera Vaccines by the infant Rabbit Test. Dutta, N.K., and Dohadwala, A.N. (*Journal of Pahtology and Bacteriology*).

Characterization of the Antidiuretic Factor in the Lysate of *V. cholerae*. Oza N.B. and Dutta, N.K. (*Indian Journal of Physiology and Pharmacology*)

Antifertility Activity of the Steriodal Oil derived from the Seed of *Abrus* (Linn) of Rats and Mice. Desai, R.V. and Rupawala, E.N. (*Indian of Pharmacy*).

Experimental Cholera Absorption of Toxin into Blood from separated Jejunal segment :Dohadwala, A.N., (*Nature*, London.)

Department of Virology

The Enteroviruses and their relation to human disease. Gharpure, P.V. Ped. Clinics of India).

Complications of therapy with Antirabic Vaccine : Nanavati, A.N.D. *Medical Journal*).

Maintenance Engineering workshop and Carpentry Section

The Maintenance Engineering Section undertakes maintenance and repairs of and plant, such as cold rooms, centrifuge machines, deep-freeze, ovens, vacuum pumps, air compressors, air conditioners and refrigerators etc.

This year, both the Desivac Plants were constantly used. The number of were 45.

The Carpenter Shop prepared varieties of crates and small furniture for all the departments.

Plant and machinery which have been imported and purchased locally were tested, installed and put into commission.

PART IV

MISCELLANEOUS

MISCELLANEOUS

(1) Critics Club

During the period under review, 30 meetings were held under the auspices of the Critics Clubs, out of which 14 were addressed by Scientists from outside. At these meetings members of the staff, scholars and research fellows of the Institute either presented original research work or reviewed topics of scientific interest. The meetings were followed by critical discussions on the subject.

The following is the complete list of the speakers and subjects:—

1. Shri Bhatnagar, R. K. ... Studies on leptospirosis in Bombay.
2. Dr. John Feeley, National Institute of Health, U.S.A. Cholera vaccines.
3. Smt. Seth, P. K. ... Respiratory virus infections as a World Health Problem.
4. Dr. Deliwala, C. V. ... Studies on oral diuretics.
5. Kum. Belgaumkar, P. H. ... Bacteriocins.
6. Shri Mehta, K. H. ... Gas chromatography.
7. Kum. Advani, M. H. ... A study of dietary pattern of Sindhi community with reference to serum cholesterol and phospholipids.
8. Dr. Stahnke, H. L., Director of 'Poisonous' Animals Research Laboratory, Arizona State University. Venomous animals of Arizona desert and cryotherapy.
11. Dr. Sidney Raphel, Professor of Microbiology, Stanford University, U.S.A. Serum globulins.
12. Professor Rosenfield, R. E., The Mount Sinai School of Medicine New York, U.S.A. Immunogenic aspects of quantitative haemagglutination.
13. Dr. Luyet, B. J., American Foundation for Biological Research, Madison, Wisconsin. Effects of freezing on muscle, on blood and on entire chick-embryo heart.
14. Mr. Payne, B. O., Technical Manager, Messrs. Vickers Instruments Ltd., York, England. Fluorescence microscopy.

15. Dr. Jhala, H. I. ... Report of the 9th International Congress of Microbiology.
16. Dr. Deoras, P. J. ... Impressions of the International symposium on venomous animals of Sau Paulo.
17. Dr. Lowenthal, J. P., Walter Reed Army Institute of Research U.S.A. Current trends in production of biologics.
18. Dr. Goodman, H. C., Chief Immunology, World Health Organisation. Biological properties of immunoglobulins.
19. Dr. Kabat, E. A., Columbia University, U.S.A. The nature of antigenic determinant.
20. Dr. Jai, V. N. ... (i) Food-*cum*-nutritional studies in Niger.
21. Dr. Jai, V. N. ... (ii) Advanced training in applied nutrition in London.
22. Dr. Peters, Heidelberg University, West Germany. Rodent control in West Germany.
23. Dr. Harisijades, School of Medicine, Belgrade, Yugoslavia. Formal discussion.
24. Dr. E. M. Vaughan Williams, Oxford University. The mechanism of action of recently introduced anti-arrhythmic compounds.
25. Dr. Lawrence, S. C. Griffith, Surgeon, U. S. Public Health Service. Pediatric study of cholera.
26. Shri R. K. Bhatnagar ... A serologic survey of animal leptospirosis.
27. Dr. A. N. D. Nanavati ... Studies on pathogenesis of rabies, role of brown fat.
28. Shri M. G. Dhapalapur ... Synthesis of potential anti-cancer agents.
29. Dr. Ernest Tierkel, Formerly in-charge Rabies Division at the C. D. C., Atlanta, Georgia, U.S.A., now working with the N. I. C. D., Delhi. Some representative zoonotic diseases.
30. Kum. Karnikar ... Breeding of *Musca sorbens* Wied and homologous and heterologous transplantation of corpus allatum in allied species of the genus *Musca*.

(2) Distinguished Visitors

1. Dr. P. Carpenter, Dysentery Reference Laboratory, Central Health Laboratory, Collindale Avenue, London, M.W.G.
2. Dr. John C. Feeley, Division of Biological Standards, National of Health, Bethesda, Maryland, 50014, U.S.A.
3. Dr. G. H. Brown, Consultant Physician (Infectious Diseases and Medicine), Hither Green Hospital, London S.E.B.
4. Dr. James M. Hundley, F.A.O./W.H.O./UNICEF Consultant Applied Nutrition.
5. Dr. R. Bubina, W.H.O., New Delhi.
6. Dr. G. Gouljaev, Docent of Institute, Postgraduation of Doctors Moscow.
7. Dr. G. Pafomov, Senior Doctor, Skeifojdusky Institute, Moscow.
8. Dr. Marilyn Hastings, Programme Economist, U.S.A.I.D., New Delhi.
9. Dr. William D. Fitzwater, UNESCO Expert, CAZARI, Jodhpur.
10. Dr. Ira Kaye, Western Regional Director, American Peace Corps.
11. Dr. Fred Wong, Principal, Training Centre for Blind, Kualalumpur, Malaysia.
12. Dr. B. Bager, N.I.C.D., Delhi, W.H.O. Consultant.
13. Shri S. V. Krishnamurthy Rao, Dy. Speaker, Lok Sabha, New Delhi.
14. Dr. Jesus MA. de. Leon, Quarantine Medical Officer, Manila, Philippines.
15. Dr. W. W. Wai, Member, Estimates Committee, Parliament of India.
16. Shri R. C. Mallick, M.P. (Orissa), Member, Estimates Parliament of India.
17. Shri B. N. Kureal, M.P. (U.P.), Member, Estimates Parliament of India.
18. Shri J. N. Hazarika, M.P., Member, Estimates Committee, of India.
19. Shri N. M. R. Subharaman, M.P. (Madurai, Madras,) Member, Estimates Committee, Parliament of India.
20. Shri B. K. Mukherji, Secretary, Study Group, Estimates Committee.

21. Dr. Sidney Haffal, Staford University School of Medicine, California.
22. Dr. P. Rozenfield, The Mount Sinai Hospital, New York.
23. Dr. B. Laget, American Federation for Biological Research, Madison, Wisconsin, U. S.A.
24. Dr. A. A. Sandosham, Kuala-Lumpur, Malaysia.
25. Dr. E. M. Vaughan William, Department of Pharmacology, Oxford University, U. K.
26. Dr. J. P. Lowenthal, Washington, D. C., U.S.A.
27. Dr. G. Roux, Glaxo International Ltd., London.
28. Shri R. C. Joshi, I.C.S., Secretary, U.D.,P.H. & H.D., Maharashtra Government.
29. Prof. Elwin G. Kabat, Columbia University of W.H.O.
30. Prof. Howard Goodman, W.H.O., Geneva, Switzerland.
31. Prof. Dr. H. Peter, Heidelberg, Germany.
32. Dr. J. Harisjidas, Institute of Microbiology, Medical Faculty, University of Belgrade.
33. Dr. Fredrick Aladjem, U. S.C. School of Medicine, U.S. Aid.
34. Dr. Lawrence S. C. Griffith, U. S. Naval Medical Research Unit No. 2. Taipai, Taiwan, Republic of China.
35. Dr. Christian Serche, Director of the National Institute of Health, OSCO, Norway.
36. Dr. John Kepling, Senior Bacteriologist, Norway.
37. Dr. W. B. Clark, Medical Officer of Health, New Fort, Mon, England.
38. Dr. Augus McGregor, Medical Officer, Southampton, England.
39. Dr. Gerald T. Kensch, SEATO Medical Research Laboratory, Bangkok, Thailand.
40. Shri Dev. Raj Mehta, Ex. W. H. O. Consultant.
41. Dr. P. B. Andrey, W. H. O. Consultant, Geneva.
42. Dr. Earnest S. Tierkel, NCDC Zoonos E Consultant, USAID (Atlanta).
43. Dr. Lane Barksdale, Professor of Microbiology, New York University Medical Centre, New York City 10016.
44. Dr. Willi Heine, Hannover, Germany.

(3) Academic Courses and Training of Health Personnel

The Institute is affiliated to the University of Bombay for post-graduate training courses for the following degrees and Diploma:—

M. Sc. and Ph. D. in Microbiology, Bacteriology, Biochemistry (including Nutrition), Zoology, Organic Chemistry and Applied Biology.

M. Sc. (Med.) and Ph. D. in Pathology, Bacteriology, Parasitology and Pharmacology.

Diploma in Medical Laboratory Technology (D. M. L. T.).

The following are post-graduate teachers recognised by the University Bombay :—

1. Dr. H. I. Jhala.
2. Dr. N. K. Dutta.
3. Dr. P. J. Deoras.
4. Dr. C. V. Delivala.
5. Dr. M. V. Sant.
6. Dr. A. N. D. Nanavati.
7. Dr. (Smt.) A. Venkataraman.
8. Shri K. G. Anantanarayanan.
9. Dr. S. S. Rao.
10. Dr. S. D. Ambegaonkar.
11. Dr. R. B. M. Colah.
12. Dr. K. H. Dave.
13. Shri R. A. Bellare.
14. Dr. K. V. Kulkarni.
15. Shri Y. S. Nimbkar.

The Institute is also recognised by the College of Physicians and Surgeons of Bombay for training candidates for (a) F. C. P. S. Examination in Pathology and Pharmacology and (b) D. P. B. Examination of the College. The following officers of the Institute are recognised as teachers of the College:—

1. Dr. H. I. Jhala.
2. Dr. N. K. Dutta.
3. Dr. M. V. Sant.

(4) No. of Post-Graduate Students on Roll 1966-67

M.Sc.

Ph. D.

Students for Organic Chemistry.

- | | |
|--------------------------|---------------------------|
| 1. Shri B. S. Fernandes. | 1. Shri M. G. Dhapalapur. |
| 2. Shri R. S. Sheth. | 2. Shri R. B. Petigara. |
| 3. Shri K. M. Thomas. | 3. Shri J. D. Modi. |
| | 4. Shri D. J. Vadodaria. |

Students for Biochemistry.

- | | |
|-------------------------|------------------------|
| 1. Shri J. K. Kamath. | 1. Shri M. J. Modak. |
| 2. Shri M. B. Borkar. | 2. Shri R. A. Bellare. |
| 3. Shri A. D. Nadkarni. | 3. Kum. M. S. K. Nair. |
| 4. Shri V. M. Patankar. | |
| 5. Shri A. M. Bijur. | |
| 6. Shri S. T. Nikam. | |
| 7. Shri T. V. Darnule. | |
| 8. Shri R. G. Tamhane. | |

Students for Microbiology.

- | | |
|-----------------------------|----------------------------|
| 1. Shri S. M. Desai. | 1. Smt. S. M. Gadgil. |
| 2. Shri K. K. Das. | 2. Kum. A. E. Bundeally. |
| 3. Kum. M. S. Kulkarni. | 3. Shri N. P. Parikh. |
| 4. Shri R. P. Bhamaria. | 4. Kum. S. K. Sardesai. |
| 5. Kum. L. S. Rao. | 5. Shri F. L. Saldanha. |
| 6. Shri K. N. Joishy. | 6. Kum. P. H. Belagaonkar. |
| 7. Shri J. M. Mehta. | 7. Kum. L. S. Iyer. |
| 8. Shri S. Jesudas. | |
| 9. Shri R. K. Bhatnagar. | |
| 10. Kum. R. F. Billimoria. | |
| 11. Shri B. T. Rawal. | |
| 12. Kum. G. V. Shirgaonkar. | |
| 13. Shri P. R. Dichwalkar. | |
| 14. Kum. P. D. Patel. | |
| 15. Kum. K. S. Bandodkar. | |

Students for Applied Biology

- | | |
|-----------------------------|--------------------------|
| 1. Shri P. G. Fernando ... | 1. Shri D. P. Buduk. |
| 2. Shri T. Unnikrishnan ... | 2. Shri A. N. Dohadwala. |
| 3. Shri S. N. Iyer ... | 3. Kum. S. M. A. Hakimi. |
| 4. Shri A. S. Parikh. | |
| 5. Shri A. A. Parelkar. | |

Students for Zoology

- | | |
|------------------------|-------------------------------------|
| 1. Shri T. G. Tare ... | 1. Shri N. E. Wad. |
| | 2. Shri G. C. Chaturvedi. |
| | 3. Kum. K. M. Karnikar (Smt. Khot). |

Students for M.Sc. (Med.) Pathology and Bacteriology

Dr. S. J. Mokashi up to 31st December 1966.

Kum. Y. Fernandes	... D. P. B.	... Pathology.
Smt. D. Chengappa	... D. P. B.	... Pathology.

(5) Candidates obtaining post-graduate degrees

Candidates who obtained degrees from 1st April 1966 to 31st March 1967

	M.Sc.	Topics
1. Shri A. K. Bhatt	... Organic Chemistry.	"Synthesis of Compounds for therapeutic value special reference to fungal Activity."
2. Kum. L. S. Iyer	... Microbiology	"Studies on pattern phase typing serological classification pathogenic staphylococci."
3. Shri V. P. Shinde	... Biochemistry ...	"Protein Metabolism 'B' Vitamins, with special reference to pathogenic acid and Vitamin B ₁₂ ."
4. Shri M. J. Modak	... Biochemistry ...	"Studies on Bacterial Metabolism."
5. Kum. Z. S. Kapadia	... Biochemistry ...	"Studies in Antibody protein of horse sera."
6. Shri K. Y. Mahajan	... Organic Chemistry.	"2-Methyl 1 and 2-Aryl pyrimidine as possible therapeutic agents."
7. Shri P. Divakaran	... Biochemistry ...	"Studies in lipid metabolism."

Ph.D.

1. Shri M. D. Gadgil	... Microbiology	"Nutrition, Growth and Morphology of <i>P. pestis</i> in relation to vaccine production."
2. Shri R. S. Prasad	... Zoology ...	"Some studies on Rat Fleas and their bearing on Host Specificity".
3. Shri S. S. Sabnis	... Organic Chemistry.	"Anti-cancer Agents."
4. Shri M. H. Shah	... Organic Chemistry.	"Diuretic Agents."

(6) Short-term Trainees

Short-term Trainees between 1st April 1966 to 31st March 1967

Name of trainee	Department
1. Dr. K. R. Datta ...	Blood Bank Department.
2. Shri J. N. Ray Biswas ...	Do.
3. Dr. C. M. Patnaik ...	Do.
4. Shri M. M. Mishra ...	Do.
5. Shri Narottam Mohanty ...	Do.
6. Dr. B. C. Das ...	Do.
7. Shri V. B. Surpuria ...	Bacteriology Department.
8. Dr. D. T. Dixit ...	Do.
9. Kum. J. P. Nair ...	Pharmacology Department.
10. Kum. Jayamala Kamath ...	Do.
11. Shri V. M. George Verghese ...	Do.
12. Shri Subodh W. Desai ...	Do.
13. Kum. Rekha M. Mehta ...	Do.
14. Shri N. M. Sathe ..	Do.
15. Shri D. L. Jadeja ..	Clinical Pathology Department.
16. Kum. R. S. Ranade ...	Do.
17. Shri B. M. Pandya ...	Do.
18. Shri S. N. Paranjpe ...	Biochemistry Department.
19. Shri Matha V. D'Mello ...	Do.
20. Kum. Bablu Bhawanani ...	Do.
21. Shri Mohan S. Rao ...	Entomology Department.
22. Kum. Rajkumari Hingorani ...	Do.
23. Shri M. L. Sultan ..	Animal House.
24. Shri D. D. Patel ...	Antirabic Department.
25. Shri C. N. Naik ...	Do.
26. Shri P. C. Gandhi ...	Do.
27. Shri Jitendra Dave ...	Clinical Pathology Department.
28. Shri N. B. Bhatt ...	Do.
29. Shri Syed A. Sattar ...	Do.
30. Shri B. D. Pandit ...	Pharmacology Department.
31. Shri V. T. Thomas ...	Do.
32. Shri M. N. Joshi ...	Immunology Department.
33. Dr. Pimpalkhare ...	Do.
34. Shri Uttamchand Sanghi ...	Bacteriology Department.
35. Shri G. Pandya ...	Analytical and Control Department.

(7) Pool Officers

(DURING THE PERIOD 1ST APRIL 1966 TO 31ST MARCH 1967)

1. Dr. Y. I. Shethna ... Department of Virology (left).
2. Dr. F. D. Bharucha ... Department of Bacteriology.

A BRIEF REPORT OF WORK DONE BY DR. F. D. BHARUCHA,
POOL OFFICER IN THE DEPARTMENT OF BACTERIOLOGY
AT THE HAFKINE INSTITUTE

"Cultivation of Micro-organisms in Deep Sub-merged Culture"

The classical method of cultivating micro-organisms for the production of bacterial vaccines, consists in growing the organisms in Roux bottles on solid media and harvesting the growth with physiological saline.

This method suffers from the disadvantages of being laborious and time consuming. We, therefore, undertook to carry out researches for the growth of *Salmonellatyphi* in liquid media under stationary, shake flask and laboratory type fermentor conditions.

Our investigation indicates that shake flask cultures gave better growth than in stationary cultures and that the method of submerged fermentation for the production of Typhoid Vaccine is commercially feasible. This technique could be subsequently used to advantage for the production of bacterial vaccines, viz. plague and cholera.

(8) Students and Trainees admitted

Students admitted during the period 1st April
1966 to 31st March 1967 for post-graduate
Studies

M.Sc. (Biochemistry)

1. Shri S. T. Nikam.
2. Shri T. V. Darnule.

M.Sc. (Microbiology)

1. Kum. G. V. Shirgaonkar
2. Shri P. R. Dichwalkar.
3. Kum. P. D. Patel.

M.Sc. (Organic Chemistry)

1. Shri K. M. Thomas.

Trainees admitted for Technicians' Training
Course from 1st April 1966 to 31st
March 1967

1. Shri R. J. Malgaonkar.
2. Kum. P. N. Paradkar (left).
3. Kum. A. K. Shah.
4. Kum. U. M. Shah.
5. Shri V. B. Shimpi (left).
6. Shri J. K. Dhorda.
7. Shri A. M. Mistry.
8. Shri G. H. Tambe.
9. Kum. M. R. Chheda.

Students admitted during the period 1st April 1966 to 31st March 1967 for post-graduate studies

M.Sc. (Applied Biology)

1. Shri A. A. Parelkar.
2. Shri A. S. Parikh.

Ph.D. (Biochemistry)

1. Shri R. G. Tamhane.
2. Shri M. J. Modak.

Ph.D. (Microbiology)

1. Kum. S. K. Sardesai.
2. Shri Francis Saldanha.
3. Kum. P. H. Belgaonkar.

Ph.D. (Organic Chemistry)

1. Shri D. J. Vadodaria.
2. Shri J. D. Modi.

D. P. B. (Pathology)

1. Dr. Kum. Y. Fernandes.

Trainees admitted for Technicians' Training Course from 1st April 1966 to 31st March 1967

Trainees admitted for Diploma in Medical Laboratory Technology (D. M. L. T).

1. Kum. A. R. Nadkarni.
2. Kum. S. B. Padhye.
3. Kum. P. D. Shah.
4. Kum. L. M. Modak
5. Kum. S. V. Kotnis.
6. Kum. S. R. Pathak.
7. Kum. R. R. Jerath.
8. Kum. K. S. Tirodkar.
9. Shri U. J. Dumanwalla.
10. Kum. S. B. Naik.
11. Kum. H. B. Shah.
12. Shri R. P. Vitkar.
13. Kum. N. H. Paigaonkar (left).
14. Shri S. V. Pethe (left).
15. Kum. S. P. Gadkari (left).

(9) Research fellowships and scholarships

The following Research Fellowships and Scholarships were attached to the Institute during the period from 1st April 1966 to 31st March 1967:—

Serial No.	Sponsors	Subject of Research Fellowships and Scholarships	Name of the candidate	Amount
1	2	3	4	5
1	Council of Scientific and Industrial Research.	For carrying out Research on the Scheme "Synthetic Diuretics".	Shri H. V. Patel from 3rd January 1966.	Rs. 492.
2	Do.	.. For carrying out Research on the Scheme "Microbiological Research".	Shri J. S. Desai from 11th January 1967.	Rs. 492 (inclusive of basic pay, D. A., C. I., A. & H. R. A.)
3	Government of India Research Training Scholarship.	For carrying out Research in "Entomology".	Shri R. S. Prasad from 23rd August 1962 to 23rd August 1966.	Rs. 250 p.m. (fixed).
4	Do.	.. For carrying out Research in "Immunology".	Shri V. D. Kadam from 9th February 1967.	
5	Do.	.. For carrying out Research in "Organic Chemistry".	Shri R. B. Petigara from 1st February 1964 to 30th October 1966.	
6	Do.	.. For carrying out Research in "Organic Chemistry".	Shri J. D. Modi from 13th December 1966.	Rs. 250 p.m. (fixed).
7	Do.	.. For carrying out Research in "Organic Chemistry".	Shri M. G. Dhaplapur from 24th June 1964.	
8	Government of Maharashtra Research Training Scholarship.		—Vacant—	.. Rs. 150 p.m. (fixed).

Serial No.	Sponsors	Subject of Research fellowships and Scholarships	Name of the candidate	Amount
1	2	3	4	5
9	Government of Maharashtra Research Training Scholarship.	To carry out Research work in "Microbiology".	Shri K. N. Joishy from 1st March 1965.	Rs. 150 p.m. (fixed).
10	Do.	To carry out Research work on "Lipid Metabolism".	Shri P. Divakaran from 4th December 1963 to 3rd December 1966.	Rs. 150 p.m. (fixed).
11	Do.	Do.	Shri S. S. Samant from 16th March 1967.	Rs. 150 p.m. (fixed).
12	Government of Maharashtra Sr. Research Training Scholarship.	To carry out Research work on "Lipid Metabolism".	Shri P. Divakaran from 21st January 1967.	Rs. 300 p.m. (fixed).
13	Unichem Laboratories Ltd...	For Research on "Cholera"	Shri A. N. Dohadwalla from 20th September 1965.	Rs. 350 p.m. (fixed).
14	Do.	For Research on Indigenous Drugs...	Kum. S. E. Bhagwager from 21st May 1966.	Rs. 350 p.m. (fixed).
15	Dorabji Tata Trust Scheme (Now Unichem Grant).	To carry out Research on problem of "Cataract".	Kum. N. G. Gogwekar	Rs. 300 p.m. (fixed)
16	Indian Council of Agricultural Research.	"Immuno Studies in Animal Viruses with special reference to Pox Group of Viruses and mucosal disease complex viruses."	Smt. Thankam Mathew from 15th November 1964 to 31st January 1967.	Rs. 250 p.m. (fixed).
17	University Grants Commission Jr. Research Fellowship.	To carry out Research in "Organic Chemistry".	Shri R. B. Petigara from 31st October 1966.	Rs. 300 p.m. (fixed).
18	I. C. M. R. Technicians' Training Fellowships.	To carry out Research work in "Microbiology".	Shri Stanley Jessudas from 14th June 1965 to 13th June 1966.	Rs. 150 p.m. (fixed).

19	Do.	..	Do.	..	Shri R. K. Bhatnagar from 7th August 1965 to 31st December 1966.	Rs. 150 p.m. (fixed).
20	I. C. M. R. (Blood Group reference Centre) One Research Fellowship.		Preparation of Blood Grouping Reagents.		Shri A. S. Parikh from 29th November 1965.	Rs. 300 p.m. (fixed).
21	Haffkine Institute Diamond Jubilee Commemoration (Research Unit) Sr. Research Fellowships.		"Biochemistry of Vibrio Cholera."		Dr. (Kum.) K. Deshmukh from 31st January 1963 to 30th September 1966.	Rs. 560 p.m. (fixed).
22	Do.	..	"Studies on Lysis of <i>Vibrio Cholera</i> ."		Dr. S. V. Huprikar from 7th December 1964 to 10th September 1966.	Rs. 530 p.m.
23	Haffkine Institute Diamond Jubilee Commemoration (Research Unit) Sr. Research Fellowships converted into Jr. Research Fellowships.				Shri S. P. Thorat 1st from February 1967.	Rs. 300 p.m.
24	Haffkine Institute, Diamond Jubilee Commemoration (Research Unit) Sr. Research fellowships.		"Studies on Toxin Producing Strains of <i>C. diphtheriae</i> ".		Dr. A. B. Rajadhyakasha from 20th February 1961 to 31st October 1966.	Rs. 500 p.m.
25	Do.	..	"Continuation of studies on <i>Rabies Virus</i> ".		Dr. (Kum.) B. A. Ver from 1st March 1961 to 17th August 1966. Now on study leave for 2 years.	Rs. 500 p.m.
26	Do.	..	(1) "Level of Measles Antibodies in child population." (2) "Experimental Infections of Lab. Animals and Enteroviruses."		Dr. M. R. Samuel, from 1st February 1967.	Rs. 500 p. m.
27	Do.	..	"Nutritional requirement of <i>P. pestis</i> and their relation to vaccine production."		Dr. M. D. Gadgil from 5th March 1966.	Rs. 350 p.m.

Serial No.	Sponsors	Subject of Research Fellowships and Scholarships	Name of the candidate	Amount
1	2	3	4	5
28	Haffkine Institute Diamond Jubilee Commemoration (Research Unit) Jr. Research Fellowships.	"Studies on <i>Bordetella pertussis</i> " ..	Kum. A. E. Bundeally from 1st July 1963.	Rs. 350 p.m.
29	Do.	.. "Biochemical Composition of <i>P. pestis</i> in relation to virulence and immunogenicity."	Smt. S. M. Gadgil from 6th July 1963.	Rs. 350 p.m.
30	Do.	.. "Studies on Hypersensitivity to Horse Serum."	Kum. S. K. Sardesai from 1st December 1964.	Rs. 325 p.m.
31	Do.	.. "Studies on Salmonella Metabolism."	Shri M. J. Modak, from 1st April 1966.	Rs. 300 p.m.
32	Do.	.. "Studies on Bacteriocine of Clostridia."	Kum. P. H. Belgaonkar from 18th April 1966.	Rs. 300 p.m.
33	Do.	.. "Studies on Measles Virus" ..	Kum. N. A. Varaiya from 30th April 1966.	Rs. 300 p.m.
34	Pfizer Scholarship (Diamond Jubilee Scholarship Unit)	To standardize the method for the Bioassay of insulin and to determine the factors affecting the method, including the action of various drugs. Thereafter to determine the insulin levels in diabetic patients.	Kum. P. Ramnathan from 26th August 1966 to 25th January 1967.	Rs. 300 p.m.
35	Haffkine Institute Diamond Jubilee Commemoration (Scholarship Unit) Unichem Scholar.	"Studies on Chemotherapy of Viral Infections."	Kum. N. A. Varaiya from 4th January 1965 to 29th April 1966.	Rs. 250 p.m. (fixed).
36	Do.	.. "Studies on the Chemotherapeutic Drugs."	Shri J. D. Modi from 17th September 1965 to 17th September 1966.	Rs. 250 p.m. (fixed).

(10) Guest Workers and Trainees

1. Prof. Vaughan Williams Guest Worker (on his own) from University Department of Pharmacology, South Park Road, Oxford, England, to carry out work on Cholera with Dr. N. K. Dutta. Department of a period of six months 2nd November 1966.
 2. Shri Habibullah Hashimi, Laboratory Technician, Public Health Laboratory, Kabul, Afghanistan, for training in Virus Vaccine Production (Through W.H.O.). Virology Department for months from 6th October
 3. Shri Georgi Dimitrov Dimitrov "Assistant", at the Institute of Chemistry, Chair of Organic Chemistry, Sofia, Bulgaria. Immunology Department for one year from 23rd March 1966 to end of March 1967.
- Training in methods of Fractionation of Snake Venoms.
- Under Indo-Bulgarian Cultural Exchange Programme.
4. Dr. J. P. Lowenthal (U. S. Army, Walter Reed Army Institute for Medical Research) W. H. O. Consultant for Acetonekilled typhoid Vaccine visited the Vaccine Project in the month of November for 10 days. Department of Bacteriology.
 5. Shri Sukchippa Taitalamanda—Trainee under Technical Co operation Scheme (Colombo Plan). Department of Pharmacology for one month from 20th March 1967.

(11) Grants-in-aid Research Schemes

(BETWEEN 1ST APRIL 1966 TO 31ST MARCH 1967)

C.S.I.R.			
Schemes	Staff	Grants sanctioned	
		Rs.	
1. "Synthetic Diuretics"	Shri H. V. Patel	Staff	.. 4,461
	(in Chemotherapy)	Cont.	.. 750
			6,875
2. "Microbiological Research"	Shri J. S. Desai	..	3,000

*I.C.M.R.**I. Pharmacological Research Unit—*

1. Research Officer	..	1	
2. Research Assistant	..	1	
3. Laboratory Technician	..	1	
4. Laboratory Assistant	..	1	
5. Animal Attendant	..	1	
Total No. of posts	..	5	
Total sanctioned grant	..		31,445

II. Preparation of Type Specific Antiviral Anti-sera—

1. Technicians	..	2	
2. Laboratory Attendants	..	2	
3. Animal Attendants	..	4	
Total No. of posts	..	8	
Total sanctioned grant	..		17,235

III. Isolation of Cholera Toxin—

1. Research Assistant	..	1	
2. Laboratory Attendant	..	1	
Total sanctioned grant	..		6,060

IV. Blood Group Reference Centre—

1. Senior Research Officer	..	1	Complement that works at Haff- kine Institute consists of 1 A.R.O., 1 Res. Fellow and 1 U.D.C. Rest work at K. E. M., Wadia and I.C.R.C.
2. Assistant Research Officers	..	4	
3. Research Assistants	..	2	
4. Research Fellow	..	1	
5. U.D.C.	..	1	
6. Technician	..	1	
7. Junior Laboratory Assistant	..	1	
8. Laboratory Attendants	..	3	
Total No. of posts	..	14	
Total sanctioned grant	..		95,205

V. *Polio Research Unit—*

Research Officers	..	3 (1 vacant)
Assistant Research Officer	..	1
Technicians	..	8 (2 vacant)
Social Worker	..	1
Health Visitor	..	1
Upper Division Clerk	..	1
Lower Division Clerks	..	2
Laboratory Assistants	..	2
Laboratory Attendants	..	7
Animal Attendants	..	6
Peons	..	2
Total No. of posts	..	34 (3 vacant)
Total sanctioned grant	..	1,32,210

(12) **Business Side**

This year also the position continued to be extremely critical for the Institute. One of the major events was devaluation which affected the prices of all our imports both towards capital equipment as well as raw materials. As far as capital items were concerned, maximum economy consistent with the needs of the Institute was attempted. Regarding raw materials, we had no option but to replenish the buffer stocks which were practically exhausted, due to non-availability of import licences in the previous year. The upward trend in the locally obtained raw material prices also continued. Another important increase in expenditure was towards Establishment, due to increase in D.A. from July 1966.

Though the general financial stringency continued, the Institute managed to come upto the sales of the previous year, they being about Rs. 78,85,000. Due to abnormal imports of Tetanus Anti-toxin, sales of this item were affected to a certain extent. We have already made representations that such imports should be banned as the country is self-sufficient in this item. During this year, we have already moved in the direction of commercial marketing of some of the Institute's products like Plasma Fractions. This is a beginning and more products are likely to be covered under similar arrangements. It is expected that these would the sale of the Institute.

The Recovery Section with the staff sanctioned by the Government has completed a year and has helped considerably in clearing the past arrears. An amount of about Rs. 4.35 lakhs for the period up to 31st March 1966 is outstanding as on 31st March 1967 from parties in Maharashtra.

In view of the recommendations of the Haffkine Institute Reorganisation Committee, an attempt was made to segregate Research from Production as far as the annual accounts of the Institute are concerned. However, this is far from sufficient and much more remains to be done in this direction. Besides, for the last few years, the raw material prices and establishment expenditure have been increasing regularly and the financial pattern has now altogether changed. However, we have not been able to prepare proper accounts analysing this impact of the various new factors affecting the financial pattern owing to staff difficulties pointed out by the said committee.

For several reasons, it may not be possible for a Government Organisation to implement sophisticated managerial techniques. Even then, certain essential functions and procedures require to be introduced. Since its inception, the Institute has been carrying on without these and the Haffkine Institute Reorganisation Committee has made certain recommendations in this connection. Government has accepted in principle appointment of some senior officers and staff. When these are made available, the reorganising will have speedy effect.

Due to economy measure it became necessary to curtail an integrated warehousing scheme for Raw Materials and Finished Products. However, a phased alternative within the immediate means of Government has been suggested.

Our position as on 31st March 1966

What we have	1965-66	1964-65	
1. Land and Buildings ..	68,06,362	62,93,512	
2. Equipments ..	58,26,411	48,85,624	
3. Library Books ..	5,94,365	5,58,312	
4. Live Stock (Ex. Gift) ..	1,45,485	76,359	
5. Stocks and Stores ..	31,15,434	28,76,776	
6. Amount to be received ..	29,00,858	31,12,619	
7. Cash and Other Balances.	5,30,418	5,60,002	
	1,99,19,333	1,83,63,204	
What we owe			
1. Depreciation Fund ..	25,95,251	22,19,376	
2. Amount to be paid ..	10,37,628	11,32,991	
3. Miscellaneous Deposits ..	1,15,559	1,64,177	
	37,48,438	35,16,544	
Net Surplus (After providing for all expenses).	7,17,801	7,93,070	
What remains (Capital less current year's profit).	1,54,53,094	1,40,53,590	

What we earned and spent in 1965-66

What we earned

1. From selling Biologicals and Drugs.	78,18,530	88,09,143	
2. From Analytical Tests ..	2,99,423	2,55,514	
3. Miscellaneous Sources ..	4,39,234	28,953	
4. Value of closing stock ..	31,15,434	28,76,776	
5. Reserve for Bad Debts ..	31,027		
6. Interest on Funds ..	1,42,955		
	1,18,46,603	1,19,70,386	

What we spent

1. On our Employees ..	24,98,474	23,93,236	
2. On Raw Materials ..	35,19,447	45,68,688	
3. On other purchases and services.	8,21,879	8,18,258	
4. Value of Opening Stock.	28,76,776	21,18,594	
5. On provision for Pension, Depreciation, Interest etc.	14,12,226	12,78,540	
	1,11,28,802	1,11,77,316	
Net Surplus (after providing for all expenses).	7,17,801	7,93,070	

Statement of supplies made by the Institute during the year 1966-67

Name of the Product	Quantity supplied	
Cholera Vaccine (Classical)	... 71,68,387 ml.	
El Tor Vaccine	... 20,37,260 ml.	
Cholera El Tor Vaccine	... 21,08,950 ml.	
T. A. B. Vaccine	... 4,82,113 ml.	
Plague Vaccine	... 95,340 ml.	
Antirabic Vaccine	... 44,10,727 ml.	
Tetanus Antitoxin	... 11,67,039 ml.	
Tetanus Toxoid	... 2,64,429 ml.	
Diphtheria Antitoxin	... 1,11,532 ml.	
Diphtheria Toxoid (P.T.A.P.)	... 9,165 ml.	
Gas Gangrene Antitoxin	.. 93,080 ml.	
Anti-snake Venom Serum	... 2,95,520 ml.	
Normal Horse Serum	... 10,060 ml.	
Triple Antigen	... 5,813 ml.	
Intravenous Solutions	... 2,58,995	} Bottles each of 500 ml.
Distilled Water	... 16,460	
A. C. D. Solution	... 4,737	

Name of the Product	Quantity supplied
Vitamin Tablets	... 849,75,692 Nos.
Sulpha Tablets	... 192,26,000 Nos.
Other Tablets (Magnesium Trisilicate and Tolbutamide).	18,38,020 Nos.
Pemon	... 17,611 litres.
Cobra Venom	... 40·16 gms.
Russell's Viper Venom	... 14·91 gms.
Krait Venom	... 6·10 gms.
Echis Venom	... 3·0633 gms.
Scorpion Venom	... 1·00 gms.
Inoculation Outfit (complete)	... 187 Nos.
Snakebite Outfit (complete)	... 63 Nos.
Dead Emulsions	... 4,49,285 ml.
High-titre sera	... 4,262 ml.
Kahn Antigen	... 15,150 ml.
W. R. Antigen	... 2,000 ml.
Casoni's Antigen	... 350 ml.
Cobra Venom Solution	... 1,502 ml.
Russell's Viper Venom Solution	... 1,030 ml.
Biological Stains	... 10,11,850 ml.
Blood Plasma	... 1,444·00 litres
Blood Grouping Sera—	
Anti-A	... 19,164 ml.
Anti-B	... 19,403 ml.
Anti-A <i>plus</i> B	... 631 ml.
Coomb's reagent	... 367 ml.
Bovine Albumin	... 799 ml.
Human Gamma Globulin, 1 ml. amps	... 2,112 ml.
Human Gamma Globulin, 2 ml. amps.	... 586 ml.
Normal Human Serum Albumin	... 20×100 ml.
Pasteurised Plasma Solution	... 20×250 ml.
Human Fibrinogen	... { 67×150 ml. 18×10 ml.

(13) Welfare Activities

General.—The welfare activities in the year under report were quite encouraging. Several indoor and outdoor games were played and tournaments were organised by the Haffkine Institute Sports Club both for outside teams as well as on inter-departmental basis.

The distribution of skim milk to the children, under the UNICEF Skim Milk Distribution Scheme in the workers' residential quarters of this Institute is continued and, on an average, 75 beneficiaries take advantage of this Scheme every day. For the benefit of the children between the age of 8 to 15 years staying in the residential quarters of this Institute, a physical training class has been started and the children are taught Dumbbells, Lezim and Drill. About 60 children take advantage of this activity.

Showing documentary films is a regular feature and the employees and their families in the Institute Compound derive benefit therefrom.

Fifteen members of the Class IV staff of this Institute were admitted to the Workers' Education Scheme, conducted by the Bombay Labour Institute (Government of Maharashtra), Parel, Bombay-12.

Collection for the following funds was arranged:—

- (1) Lal Bahadur Shastri Memorial Fund.
- (2) Flag Day on 7th December 1966 for the benefit of Ex-Servicemen for providing amenities to service people of our country.
- (3) Flag Day for the February 1967.

For the benefit of the ladies and children of the employees of this Institute's Farm at Pimpri, Haffkine Institute Farm Mahila Vikas Mandal, Pimpri, was formed during the year under report. Literacy Class, Sewing Class and training in the upkeep of Poultry Birds was started under the guidance of Directress, Mahila Vikas Mandir, Poona. The Haffkine Institute Farm Sport and Entertainment Centre, Pimpri, was started and the following indoor and outdoor games were played:—

Carrom, Chess, Volley Ball.

Some of the important days of national festivity were celebrated in the residential quarters of this Institute and full-length films were shown and entertainment programmes like Mimicry, Light, Devotional and Film Music, Kavya Vachan, Record Dance, Samargeets and Lokanatya by famous artistes were arranged for the benefit of the employees and their families, particularly residing in the residential quarters.

(14) Haffkine Institute Co-operative Credit Society Ltd.

The Committee have the pleasure to submit the satisfactory Annual Report of the Society ending 30th June 1966.

The committee have recorded its deep sense of sorrow at the sad and premature demise of our beloved Late Prime Minister, Shri Lal Bahadur Shastriji.

The total strength of the Society as it stood on 30th June 1966 is 650.

The total subscribed capital as it stood on 30th June 1966 has gone up to Rs. 3,24,280·69.

The loans advanced during the year amounted to Rs. 3,02,518·70.

Appropriation of Profits

			Rs.
Reserve Fund 25 per cent.	...	...	... 3,483·82
Divisional Equalisation Fund	...	...	... 300·00
Charity Fund	...	...	... 50·00
Dividend at 3-3/5 per cent.	...	...	... 11,000·00
Bonus to staff	...	...	... 518·00
Balance	...	...	... 31·55
			15,383·37

Dispensary

The Institute dispensary continued to serve the needs of the staff of the and their dependants as usual. A full-time Pharmacist is now in of dispensing. This has facilitated the work of the dispensing greatly services of Blood Bank Staff Nurse are utilised in attending to female and to help in giving injections.

A new more specious room has been allotted for the Clinic. The department Blood Bank and Clinical Pathology co-operated in running this service. requiring expert guidance were referred to the teaching hospital.

The number of patients attended to was 27,000.

