
ANNUAL REPORT
HAFFKINE INSTITUTE

1964-65



GOVERNMENT OF MAHARASHTRA

PRINTED AT THE GOVERNMENT CENTRAL PRESS, BOMBAY-4 : 1966

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THE HAFFKINE INSTITUTE, BOMBAY

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Dr. A. E. Kulkarni, G.B.V.C. (BOM.), Senior Scientific Officer, in charge, Animal Farm.

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Shri P. K. Sholapurwala, B.SC. (BOM.), LL.B. (BOM.), Business Manager.

Shri B. O. Shah, B.COM. (BOM.), Cost Accounts Officer, upto 30th July 19

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

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Shri B. A. Sakhardande, Labour Welfare Officer.

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

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PART I

GENERAL REVIEW

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1964-

GENERAL REVIEW

Almost as the year commenced, cholera broke out as an epidemic in city of Bombay which was free from this disease for over a decade. Cholera in the mofussil, which raised its head since November 1963, had subsided. This had resulted in severe strain in the working of the Institute. Enough vaccine was supplied to all parts of the State and a temporary stock pile was created by obtaining vaccine from other Institutes. The year also marked the holding of the International Eucharistic Congress Bombay in November-December 1964. Elaborate precautions were taken against the spread of cholera during the Congress, which had passed uneventfully.

The need for emergency supplies of dry plasma continued as before and the response of public donation of blood has not kept pace with need. A meeting of all officers of various States participating in the production of dry plasma in the country was called by the Central Government at this Institute with a view that the installations of the plasma plant be expedited. Several problems were discussed and the working of plant was demonstrated. During the year, the old Desivac Plant had to be put out of commission for some time, as it required complete overhauling for its efficient working.

The Institute continued to perform its functions as hitherto, but increasing tempo of sales had to be counterbalanced by readjustments of the available resources. The training programmes were continued. Progress of Third Five-Year Plan Schemes was satisfactory.

Dr. N. K. Dutta, Assistant Director, Department of Pharmacology, awarded the Degree of D.Sc. by the Oxford University for his valuable contributions done at the Institute. He participated in the W.H.O. Cholera Expert Group Meeting at Manila, Philippines, and Cholera Research Symposium sponsored by the East and West University of Hawaii, held Honolulu. Kum. M. S. Kotnis and Dr. (Kum.) Arora returned after completion of their training in Nutrition in London and Nigeria. Dr. K. Dave returned from W.H.O. Fellowship after training in epidemiology and vaccine production for poliomyelitis. Shri V. Y. More and Shri C. Karkhanis returned after their training in plasma fractionation from

Netherlands Red Cross Institute, Amsterdam. Shri R. A. Bellare returned after study at Pasteur Institute, Paris, under the French Government Scholarship.

It needs to be recorded that the Institute lost the services of some of the senior men during the year. Dr. K. D. Kulkarni, who was associated with Serum and Chemotherapy Departments for a long time, left the services of the Institute. Shri A. G. Inamdar, who was working as Secretary-Stenographer to the Director for the last 16 years, also left the Institute for better prospects. It is gratifying to note that Dr. E. Ganapathi was appointed as Director, Regional Research Laboratories, Jammu, Kashmir, under the C. S. I. R. Shri B. O. Shah, Cost Accounts Officer, left the services of the Institute for better prospects. The Institute is poorer for all the loss of senior men.

The World Health Organisation made research grant at the Institute to Dr. N. K. Dutta for working on El Tor Vibrio. Dr. H. I. Jhala completed a fortnight's visit to Institutions in U. S. S. R. at the invitation of Russian Government. Madam Professor P. A. Vershilove and Professor V. D. Soloviev from the Institute of Microbiology and Epidemiology of the U. S. S. R. Academy of Medical Sciences in Moscow, paid exchange visits for a fortnight at this Institute. Guest trainees have been received from U. A. R., Burma and Czechoslovakia. Members of the staff continued to serve on the I. C. M. R., I. C. A. R., C. S. I. R., and other research bodies. Dr. S. S. Rao, was invited by the Institute of Science, Bangalore, for delivering a series of lectures and demonstrations. Members of the staff delivered lectures at various scientific meetings held in Bombay. Dr. S. S. Rao was invited to lecture at the University of Baroda. Dr. N. K. Dutta was invited to deliver Dr. Nathubhai Patel Lecture at the G. S. Medical College, Bombay.

To meet the recommendations of the Drugs Control Administration, a number of additions and alterations were carried out in the production sections. An attempt has thus been made to scale up the physical standards of production laboratories, despite the fact that our buildings are not primarily designed for biologic production. The construction of Diamond Jubilee Hostel and Oral Polio Vaccine Building is in progress. The new Animal House has been occupied. Difficulties regarding water supply, cold storage space and warehouse facilities continued. More stables have been built at Pimpri. A granary has also been completed. The Pimpri Farm has now been fenced properly.

The Diamond Jubilee Endowments of the Research Unit and Scholarships have been functioning for some time and some of the work appears to be yielding promising results. Some of this work was presented before the International Cell Biology Seminar held in Bombay. One of the Senior Research Fellows, Dr. A. N. Inamdar, left the Institute, after completion of his work, for placement abroad.

The Haffkine Institute Reorganisation Committee held several meetings during the year. The work of the Committee is likely to be complete and the Committee is likely to suggest a number of changes which will be of far-reaching value for the future progress of the Institute. This is the first time in the history of the Institute that a thought has been given to its reorganisation in conformity of the recent trends. The Committee made an elaborate study of the several problems facing the Institute over a period of two years and their suggestions will receive consideration from the Government.

Amongst the important research work conducted by the Institute may be mentioned the investigations in the induction of toxin in diphtheria bacilli. These researches have a bearing on the epidemiology of diphtheria in the conversion of non-toxigenic and non-virulent strains into toxinogenic virulent strains with the help of suitable phages containing the $T+$ factor. The work has also shown the possibility of breeding strains in the laboratory suitable for producing large amounts of toxin for the preparation of potent diphtheria prophylactics. The work on lens proteins also reached an important stage. Preliminary results on the prevention of galactaract in rats by 10 per cent. urea in the diet have shown promise. A trial jointly sponsored by the Department of Technical Co-operation, U. S. and the Maharashtra Government for testing the efficacy of combined active and passive immunization in patients with tetanus-prone injuries is nearing completion. The field trials on adsorbed tetanus toxoid are in progress. Combined diphtheria and tetanus toxoid in adsorbed form has passed laboratory tests. The use of protein-free media in the production of tetanus toxoid and enzyme digestion of the anti-diphtheria serum is likely to lower the reaction rate to some extent. The Institute continues to participate in the W.H.O. Cholera Vaccine Field Trial under the auspices of the I. C. M. R. at Calcutta. It also participated in International Assays of the cholera vaccines used in field trials.

The Institute was called upon to investigate epidemics of poliomyelitis in Cuttack and plague in Madras State. The work on the pathogen

of cholera has shown some interesting results. In the host there appears to be a competition between the vibriolysate produced and its degradation by the intestinal enzyme (lipase or phosphorylase). When the lysate level gains the upper hand, it acts locally as well as systematically resulting in cholera. The remarkable property shown by the anti-live vibrio sera suggests that vaccine prepared from live cultures may be of value in humans as an immunizing agent against cholera. The method of standardizing cholera vaccine in infant rabbits is under study. The kinetics of the rabies virus in hamster kidney tissue cultures have been studied. The K. F. D. Vaccine has passed all safety tests and its study on human volunteers is under progress.

The demand for cholera vaccine was very heavy. The demand for plague vaccine was mainly received from Madras and Andhra Pradesh. The targets set for production schemes under the Third Five-Year Plan in the year have been fulfilled. The production of sulpha tablets had to be curtailed on account of non-availability of imported raw materials.

During the year under review, the Bacteriology Department produced 13.8 million ml. of vaccines and issued 21.7 million ml. of vaccines. The Chemotherapy Department supplied 126 million tablets of vitamins, sulpha and other drugs and 25,433 litres of Pemon. The Entomology Department extracted 156.13 g. of venoms and supplied 141.17 g. The Department of Immunology manufactured 2,100 litres of concentrated sera and 920 litres of Diphtheria and Tetanus Toxoids. It also produced 70 litres of Triple Antigen. The Virology Department produced 5.3 million ml. of Antirabic Vaccine. Nearly 2,500 doses of oral polio vaccine are dispensed every Saturday morning at this Institute. The Institute also stocked, processed and supplied oral polio vaccine on behalf of the Ministry of Health. The Blood Bank bled 11,460 donors and produced 967 litres of dried plasma. The Media Section, besides supplying all media, also manufactured over 1,60,000 litres of parenteral solutions. The detailed figures are given in the body of the report.

The Government Analysts at this Institute tested 3,691 samples of drugs sent from various sources for analyses. Out of these, 2,197 samples were examined under the Drugs Act, but only 14 per cent. were of non-standard quality. It is gratifying to note that the number of drugs declared as of non-standard quality is decreasing as sampled from market. The Clinical Pathology Section continued to offer diagnostic service of advanced level.

Besides other tests, 2,248 samples of urine for pregnancy test, 1,1 samples of blood culture for typhoid and 4,400 samples for histology w referred to this Department. There was a huge demand for supply Diagnostic Reagents. The newly started Analytical and Control De ment carried out nearly 36,000 tests of different samples of finished bi logical products manufactured by the various departments of the Institu

During the year under review, four Pool Officers were posted at t Institute by C.S.I.R. Nineteen short-term trainees and 11 Technic Training Course Trainees completed their courses. There were 24 grad students working under different Fellowships and Scholarships at Institute. The mice colony received slight set-back due to incidence salmonellosis. The Critics Club, Library, Dispensary, Co-operati Society and welfare activities continued with vigour. There were important foreign visitors at the Institute. Sixteen science graduates w admitted to the post-graduate courses during the year and 12 scie graduates obtained their M.Sc. and 2 students qualified for Ph.D.

The Haffkine Institute Research and Development Fund Commit supervised the functioning of the Diamond Jubilee Research Unit. State Advisory Board of the Institute continued to supervise the progr of research and developments at the Institute. Director is grateful to the members of these two committees and to their Chairmen for their tinuous assistance. Director also wishes to thank the granting bodies their generous grants, and to members of the staff for their co-opera and hard work.

PART II
SCIENTIFIC

ANALYTICAL AND CONTROL DEPARTMENT

The Department continued its work in the second year mainly in two rooms allotted to it along side the Pharmacology Department.

Shri R. G. Tamhane took over the charge of Senior Scientific Officer this Department.

In addition to the testing work undertaken last year, the Department started testing for potency and other specifications, for the Antitoxins and Toxoids manufactured by this Institute.

The Department has set up procedures to estimate preservatives such as formaldehyde and phenol in the Vaccines and Antitoxins.

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

Name of the test	Name of product	No. of samples tested
1. Sterility	(i) Bacterial Vaccines.	12,480
	(ii) Antirabic Vaccine.	11,422
	(iii) Parenteral Bulk Solutions.	4,537
	(iv) Antitoxins, Toxoids.	5,313
	(v) Miscellaneous ..	637
2. Pyrogens	Parenteral Bulk Solutions.	807
3. Chemical and Physical Examination.	Parenteral Bulk Solutions.	725
4. Abnormal toxicity	Sera and Vaccines	803
5. Absence of Infective virus.	For Antirabic Vaccines.	86
6. Potency	(a) Tetanus Antitoxin	22
	(b) Diphtheria Antitoxin.	4
	(c) Antivenine	4
7. Formaldehyde content.	In Vaccines	463

DEPARTMENT OF BACTERIOLOGY

GENERAL

During the year under review, the post of M.O., M.M.S. Cl. II was converted into a post of S.S.O. Cl. II and Shri R. S. Karbhari, J.S.O., of the department was promoted to that post. Two additional posts of Senior Technical Assistants were sanctioned and appointments were made.

The routine activity of the production of different vaccines continued as before. Investigations into the standardization of cholera vaccine by mouse protection tests were continued. The production of agar-grown vaccine which can be standardized on the basis of the number of organisms, was taken up using the casein hydrolystate as a basal medium. This was started in May and has been continued till the end of the year.

RESEARCH

The investigations on the problem of mouse protection test for cholera and plague vaccine were continued. Different aspects were examined in greater details (i) with a view to select a susceptible mouse strain; different strains of mice were screened for susceptibility for plague and cholera. Only 4 strains proved to be as susceptible as the Swiss strain which is in use at the Institute. However, irregularity of results was observed with these strains of mice also. A strain of mice in use at N. I. H., Bethesda, which is reported to be very susceptible to *Vibrio cholerae* was obtained and is being bred. Preliminary experiments with this strain show encouraging results.

(2) Six newly isolated strains of *V. cholera* Inaba and six of Ogawa were obtained from Cholera Research Centre, Calcutta. Two of the Inaba strains and five of Ogawa strains were shown to be virulent and probably suitable for our use in screening test. The detailed study of these strains is in progress.

(3) T. A. B. Vaccine : The study of locally isolated *S. typhi* strains for production of anti-typhoid vaccine is undertaken. Vaccines using three locally isolated strains along with the standard strain Ty₂ are prepared using two methods :—(i) Our usual method of formalin killed and preserved with phenyl mercuric nitrate and (ii) Heat killed and preserved with phenol.

A rabbit skin test for studying the antigenic property of these vaccines is being investigated. The M.R.D. of live *S. typhi* organisms is worked out and application of this test either as active protection or by passive protection method is under study.

Comparative study of different methods determining the antigenic property of the vaccines, viz. (i) Agglutination, (ii) Rabbit skin test (iii) Mouse protection test is undertaken.

(4) Attenuation of *V. cholera* : With a view to produce a live vaccine the attenuation of *V. cholera* strain by cultivating the organism under different cultural conditions is undertaken. Our fully virulent Inaba strain having an LD₅₀ of about 300-500 organisms in mice has been attenuated to a level where the LD₅₀ of the strain is increased to a 5 to 10 million organisms. The studies are in progress.

(5) Lysis of *V. cholera* : The study of autolysis of *V. cholera* is undertaken with a view to understand its mechanism and inhibit the lysis of vibrios during growth in broth or in suspensions of agar-grown cultures in order to evolve better control methods in vaccine production. The inactivation at different temperatures and presence of certain cations has been shown to affect the lysis. The practical application of these results and further study on related problems is in progress.

ROUTINE

The quantities of different vaccines produced and supplied are given below :—

Production

	Quantity produced ml.	Quantity ml.
Plague Vaccine	... 119,280	250,280
Cholera Vaccine	... 13,199,778	21,235,433
T. A. B. Vaccine	... 738,265	367,428

The demands of Plague Vaccine were received mainly from Madras and Andhra Pradesh.

The demands for cholera vaccine were received from the States of Maharashtra, Gujarat, Mysore and Kerala. The demands for T. A. B. Vaccine were mainly localised to Maharashtra and Gujarat States.

MEDIA SECTION

Routine.—The Media Department continued the manufacture of media, sera and reagents, required for diagnosis of diseases and for testing of bugs.

The manufacture of parenteral solutions also continued in full swing. A new product '1/6th Molar Sodium Lactate Injection B.P.' has been introduced this year. In addition to the above, the department provided general facilities for sterilization, cleaning of glassware, etc. The ampoules section supplied sterilized ampoules to Departments of Bacteriology, Immunology and Virology Department. The production figures of the different items are presented below :—

Products	Quantity manufactured
<i>Production of Culture Media</i>	
1. Casein Hydrolysate Cholera	... 12,697 litres.
Do. do. Plague	... 298 litres
2. Meat Digest Agar (Typhoid)	... 2,067 litres
3. Agar Medium for Cholera Research	... 900 bottles of 200 ml. each.
4. Sterility Media	... 1,85,580 bottles of 80 ml. each.
<i>Production of Parenteral Solutions</i>	
1. Glucose Saline (500 ml.)	... 64,766 litres
2. 5 per cent. Glucose Solution (500 ml.)	... 44,709 litres
3. 25 per cent Glucose Solution (500 ml.)	... 441 litres
4. 25 per cent. Glucose Solution (100 ml.)	
5. 50 per cent Glucose Solution (100 ml.)	
6. Normal Saline (500 ml.)	... 28,201 litres
7. Hypertonic Saline (500 ml.)	... 10,687 litres
8. Distilled Water (500 ml.)	... 10,327 litres
9. Sodium Citrate Solution (500 ml.)	... 48 litres
10. A. C. D. (500 ml.)	... 1,058 litres

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

Products	Quantity manufactured in litres
<i>Media and Solutions</i>	
1. Peptone Water	215
2. Nutrient Broth	323
3. Sterility Media (acid broth) (cooked meat media) in test-tubes	20
4. Nutrient Agar	730
5. Meat digest Agar	2,067
6. Hartley's Broth	6
7. Mac Conkey's Medium	37
8. Leoffler's Medium	42
9. Lowenstein and Jenson's Medium	19
10. Sabouraud Medium	61
11. Glucose Broth	38
12. Sugars for biochemical tests :—	
(a) Glucose	22
(b) Lactose	33
(c) Sucrose	20
(d) Mannite	22
(e) Maltose	3
(f) Arabinose	1
(g) Xylose	1
(h) Dulcit	21
13. B.S.A. Medium	41
14. Taurocholate Medium	27
15. Citrated Saline	5
16. Lemco. broth	37
17. D.E.C. Medium	23
18. Glucose Phosphate	5
19. Normal Saline	16,690
20. Selenite F. Medium	36
21. 17 per cent. Sodium Citrate	1,200

Products	Quantity manufactured in litres
<i>Stains and solutions</i>	
1. Benedict's Solution (qualitative)	... 429
2. Albert's Stains No. I	... 6
3. J. S. B. Stain No. I	... 35
4. J. S. B. Stain No. II	... 24
5. Carbol Fuchsin Ziehl Nelsen's Stain	... 54
6. Carbol Gention Violet	... 6
7. Gram's Stain No. II	... 9
8. Hayem's Fluid for R. B. C.	... 39
9. Methylene Blue (Aqueous)	... 73
10. Oxalic acid solution for W. B. C.,	... 15
11. Basic fuchin	... 27

DEPARTMENT OF BIOCHEMISTRY AND NUTRITION

GENERAL

The Department of Nutrition and Biochemistry carried out the usual manifold activities during the year under review. The laboratories continued to render effective service to the local hospitals and attend to some special requests whenever necessary. It is gratifying to note most of the Government Hospitals are now able to carry out simple routine tests for themselves and refer only some special advanced investigations to us.

Shri A. R. Advaney and Shri V. P. Shinde were transferred to the department to officiate as J.S.Os. while Shri N. R. Rao, Statistician, was promoted

Class II Officer at the Institute. Two more Senior Technical Assistants were recruited. Dr. (Smt.) Vakil continued to work as a Pool Officer under the C. S. I. R., but left in February 1965 to take up a permanent assignment in the Atomic Energy Establishment. The research scheme on "Studies in lipid metabolism" was continued. Shri Adhikari, Senior Research Fellow, however, resigned to join the Central Fisheries Laboratories. Kum. N. J. Shah was awarded the M.Sc. degree.

The Field Unit continued to carry out the activities in human nutrition and applied dietetics such as education and extension services and teaching and in practical nutrition at various levels.

Kum. Kotnis and Dr. (Kum.) Arora, Senior Nutritionists in the Department were deputed for eight months to London and Ibadan to undergo an advanced course in Nutrition and Food Sciences under an International Programme.

Smt. A. George was sent to Nutrition Research Laboratories for the months' training course.

RESEARCH

1. *Studies in bacterial metabolism.*—(a) A study of the cellular constituents of *P. pestis* (E. V. strain) and their variation with incubation time and temperature and growing conditions—stationary and shaking.

The organisms grown under stationary and shaking conditions at 27°C and 34°-36°C for 24 and 48 hours were quantitatively processed for proteins (protoplasmic building units), RNA (protein synthesis), DNA (Genetic control), total sugars including that of nucleic acids and lipid (lipid phosphorus).

Although it is reported that this organism grows much better at 27°C, significant variation could be observed as regards the above constituents under stationary conditions. However, when the incubation period prolonged from 24 to 48 hours, the RNA content seemed to diminish. Maximum synthesis of all the constituents was observed under shaken conditions for 24 hours at 27°C. The pattern of the cellular constituents when grown for 24 hours under stationary conditions was found to be similar to that observed for 48 hours with shaking.

(b) A study of the cellular constituents of the *Salmonella* organisms and the variation with incubation time (24, 48 hours) and growth medium—nutrient broth and nutrient agar.

In the case of the *Salmonella* organism, the variations were studied using two media namely nutrient broth and nutrient agar. The cells were grown under stationary conditions at 37°C. No difference was observed when the 3 organisms were grown in nutrient broth for 24 hours, but at 48 hours *S. paratyphi B* showed a lower content of nucleic acids. Compared to 24 hours old culture, 48 hours old culture showed a lower content of all the constituents except proteins. When compared to nutrient broth, growth on nutrient agar was observed to give less of RNA both at 24 and 48 hours.

(c) Incorporation of glucose-U-C¹⁴, Adenine-8-C¹⁴ and Guanine-8-C¹⁴ by the 3 *Salmonella* organisms :

It was observed that glucose was well metabolised by the 3 organisms and the activity could be determined in all the components—lipids, nucleic acids, proteins and cell-wall-polysaccharides. Although the initial amount of incorporation into the cells differed in the 3 organisms, the pattern of activity in the fractions was more or less the same. The actual distribution of the activity in the simpler components—RNA, DNA, fatty acids, glycerol, amino acids and sugars of the cell wall have been determined. The Hexokinase levels in the 3 organisms have been studied to correlate the metabolism of glucose in them. Guanine was observed to be incorporated into the cells much better than adenine—the incorporation being mainly in the nucleic acid fractions which were further fractionated into the two components RNA and DNA for locating the activities.

(d) Quantitative study of the cellular constituents of *Salmonella paratyphoid A*, *Salmonella para typhoid B* and *Salmonella typhosa*—proteins, nucleic acids, sugars and lipids at various intervals of growth.

Salmonella paratyphoid A and B and *S. typhosa* were grown in (i) nutrient broth and (ii) Kaufmann's synthetic medium at intervals ranging from 6-24 hours under suitably controlled conditions. Since RNA is an index of protein synthesis—the ratios Protein : RNA were compared. While the protein synthesis increased with incubation time the RNA content, however, was observed to reach the maximum quite rapidly at 6 hours, indicating thereby that the rapidly dividing cell population was highest at this interval. On the other hand, the DNA which is concerned with the genetic control and the process of coding of amino acids for the protein synthesis, through the RNA, was found to be more or less constant from 6-24 hours. The sugars from the cell-wall-polysaccharides after correcting for the sugar moieties of the nucleic acids did not seem to show much variation with incubation time. Lipid synthesis as shown by lipid phosphorus analysis was observed to be maximum at 6 hours with subsequent slowing down.

(e) Incorporation of labelled Xylose, Phenylalanine, and Leucine by *Salmonella* group organisms, viz. *S. para typh. A*, *para typh. B* and *S. typhosa*.

Xylose is known to stimulate the growth of all organisms in general. Leucine and phenylalanine are reported to be the growth factors for the *Salmonella*. Hence the metabolism of these three compounds was studied using radio-actively labelled compounds.

Xylose-1-C¹⁴.—Of the 3 organisms studied, *S. typhosa* showed the maximum incorporation (as high as 62 per cent), while *S. para typhi A* was found to incorporate the same very little (as low as 11 per cent.) The distribution of the activity was determined in all the cellular components—nucleic acid, cell-wall-residues, and lipids. It was interesting to note that the activity in the nucleic acid fraction of *S. para typhi A* was four times greater than that of *S. para typhi B* and *S. typhosa*. Actual distribution of the ¹⁴C carbon was studied by fractionating the nucleic acids into RNA and DNA and the lipid fraction into fatty acids and glycerol, and the residual cell into amino acids and sugars.

(f) Metabolism of leucine and phenylalanine :

The incorporation of these amino acids in general was observed to be rather low with very little activity in the nucleic acid and lipid fractions. However, the major portion of the incorporated activity could be located in the residual cell mass only, particularly in the neutral and acidic acid fractions. To support this observation the transaminases were studied and high levels were observed in the cytoplasmic material.

2. *A Critical study of the conditions for the estimation of SGO colorimetric methods.*—(a) Estimation of small amounts of pyruvic acid in the presence of ketoglutaric acid using dinitrophenylhydrazine.

To determine micro quantities of pyruvic acid by the colorimetric method it is necessary to have (i) minimum amount of the substrate—ketoglutaric acid enough DNPH to react with both the substrate and the pyruvic acid found in the reaction (ii) toluene extraction is necessary to selectively separate the hydrazones from the mixture (iii) to develop the colour with the toluene layer, it is not necessary to use alcoholic KOH as originally employed but aqueous NaOH is able to re-extract the hydrazone from the toluene phase.

3. *A study of the conditions for the estimation of serum transaminases.* While it is observed that more or less uniform procedure is followed in the case of spectrophotometric method, no effort has been made to standardise the conditions for the colorimetric assay of transaminases. Concentration of the ketoglutarate in the substrate, time and temperature of enzyme reaction, the quantity of serum and concentration of the DNPH to form the hydrazones, selective isolation of the pyruvic acid hydrazones.

by suitable solvent extraction and the development of the color with alkali are some of the factors which have to be made uniform to put the colorimetric assay on a sound footing. Expression of the results in terms of pyruvic acid units under specified conditions has to be made uniform to avoid confusion.

4. *Evaluation of proteins in foodstuffs.*—Dried seeds of 'gawar' (*Cyamopsis tetragonoloba*).—Biological evaluation of proteins of dried seeds of gawar was carried out on albino rats. It was pointed out earlier that unautoclaved material (20 per cent protein level) produced growth inhibition and mild anaemia. Hence this group was omitted in the subsequent experiment.

The growth-promoting effect and the effect on regeneration of red blood cells, haemoglobin and plasma protein of different diets containing (i) Autoclaved material (ii) Autoclaved material plus 1 per cent. dl-methionine supplement and (iii) Casein (control), were studied for a period of 8 weeks. The protein was fed at 10, 15 and 20 per cent. level in the diets. The results indicate that (i) the animals did not show any growth on diet containing 10 per cent. protein from 'gawar', (ii) the protein efficiency ratio was almost the same at 15 per cent. and 20 per cent. protein level, (iii) Methionine supplement had no effect on P. E. R. of 'gawar', (iv) the P. E. R. (1.11) of 'gawar' is significantly lower than that (2.05) of casein.

The assay of protein in 'gawar' seeds using *Tetrathymena pyriformis* W. has now been taken up.

Extraction and estimation of different fractions of nitrogen complex of 'gawar' seeds :—The fat-free material was extracted separately with water, 5 per cent. sodium chloride, and 0.25 per cent. sodium hydroxide. The results show that the fractions corresponding to 30 per cent, 66 per cent, 74 per cent, of the total nitrogen present in the meal are soluble in water, sodium chloride and dilute alkali, respectively. Fractionation of proteins in the defatted material by successive extraction with different solvents were also carried out. The isolation of the purified albumin, globulin, glutalins and other protein fractions and the study of their amino acid composition and electrophoretic pattern are in progress.

Amino-acid composition :—The essential amino-acids content of the isolated protein of 95 per cent purity (prepared by extraction with dil. alkali) was determined by microbiological method.

5. *Vitamin A and Protein Metabolism.*—The work was undertaken to study the rate of absorption and storage of vitamin A in liver and blood constituents in rats, using casein and soyabean protein. The effect of addition of choline to soyabean diet on the metabolism of vitamin A was

also undertaken. The rats were fed 20 per cent. casein, 20 per cent. soyabean protein and 20 per cent. soyabean protein plus 0.2 per cent. choline for 5 weeks, with and without vitamin A supplements. The total nitrogen, non-protein nitrogen, urea nitrogen and glutathione were determined in blood.

Effect of glutathione biosynthesis in the liver : It is observed that the total glutathione content of blood and liver is significantly lower in Vitamin A deficient rats compared to the normal. It is further planned to study the effect of Vitamin A on *in vitro* glutathione synthesis in liver homogenates using the three component amino acids namely glycine, cysteine and glutamic acid.

6. *Effect of dietary fat on cholesterol and phospholipid Metabolism.* For groups of weanling rats were maintained on four different diets, (i) 20 per cent. Safflower oil supplemented with vitamin E (1 mg. orally/day), (ii) 20 per cent. Safflower oil alone, (iii) 20 per cent. Coconut oil supplemented with vitamin E, and (iv) 20 per cent. Coconut oil alone. The results of feeding the two oils for 8½ months indicate that (i) the serum cholesterol level in rats fed coconut oil with or without supplements of vitamin E is higher than those fed safflower oil, (ii) the ratio of cholesterol to lipid phosphorus (C/P) is also slightly higher in rats fed coconut oil, (iii) the values for total cholesterol to phospholipids in liver and carcass are significantly higher in rats fed safflower oil with or without vitamin supplements, (iv) the percentage of esterified cholesterol in the carcass is about 80 to 90 in the case of safflower oil, while in the case of coconut oil it is only 6 per cent; (v) the percentage of saturated fatty acids in the carcass of rats fed coconut oil is significantly higher than that in rats fed safflower oil.

The fractionation of liver fat is in progress. Feeding experiments have been undertaken to study the cholesterol esterase activity in different tissues of rats fed coconut and safflower oils at 20 per cent level.

7. *The optimum level of dietary fat for growth, retention of protein and calcium in albino rats.*—In these experiments, the pair-fed rats were given the same amounts of total calories and protein consumed by the rats *ad lib.* a diet containing 50 per cent. calories from fat; but the amount of calories from fat were given at 30 per cent, 20 per cent, and 10 per cent levels of total calories. The feeding experiments were conducted on young growing rats for six weeks. The results indicate that if the ratio of calories from dietary protein to total calories is kept constant, the variation in amounts of calories from fat from 10 to 50 per cent. do not alter bodyweight, carcass fat, nitrogen, calcium and phosphorus.

8. *Proteins metabolism and B-vitamins.*—Experiments were carried out to study the effect of pantothenic acid and vitamin B₁₂ on protein metabolism. Four groups of rats were maintained on four different diets, viz. (i) deficient in pantothenic acid and vitamin B₁₂, (ii) supplemented with PA and B₁₂, (iii) supplemented with PA only, and (iv) supplemented with B₁₂ only. In another set of experiments, the animals were given the same diet described above, but 1.5 per cent succinylsophathiazole was included in the diets to suppress the intestinal synthesis of vitamin B₁₂. More pronounced vitamin B₁₂ deficiency was produced in this experiment, as could be seen from the lower level of liver and serum vitamin B₁₂ than that obtained in the former experiment.

The following observations have been made : (i) PA decreases the vitamin B₁₂ content of liver and serum and increases the CoA activity, (ii) vitamin B₁₂ has PA sparing effect in PA deficiency only but this effect is not seen when adequate amount of PA is present in the diet, as could be seen from the liver PA and CoA activity in liver, (iii) Vitamin B₁₂ elevates the level of RNA in the liver, (iv) PA has reverse effect. It decreases both RNA and DNA in liver. It appears that CoA and nucleic acids compete for the available ribose and adenine for their synthesis ; (v) the role of pantothenic acid in the regeneration of blood constituents such as red blood cells, white blood cells and blood proteins is more predominant than that of Vitamin B₁₂.

FIELD STUDIES

Diet-cum-Nutrition Surveys.—The State Government has recently decided to implement a programme of Applied Nutrition in selected rural areas (about 10) with 'extension' facilities. As a preliminary to the actual training of various functionaries in practical nutrition work, assessment of nutritional health by study of dietary habits and the pattern of malnutrition has been undertaken. Details of the work, which is in progress, are given below :—

Sr. No.	Village	Taluka	District	Month/Year
1	Arnala	.. Bassein	.. Thana	October 1964.
2	Gokhivare	.. Do.	.. Do.	.. Do.
3	Jamb	.. Parbhani	.. Parbhani	.. December 1964
4	Paidgaon	.. Do.	.. Do.	.. Do.
5	Dhamni	.. Ambegaon	.. Poona	.. January 1965.
6	Peth	.. Do.	.. Do.	.. Do.

In each village, 25 families have been covered. Daily variation in dietary intake being meagre, accurate data could be collected by pay two/three visits to each family. The Medical Nutritionist has completed clinical nutrition assessment in the Ambegaon Taluka (Dist. Poo). Recently, he has initiated the collection of dietary data by questionnaire method, in the Hingna Taluka (Dist. Nagpur); moreover, the Public Health Officer, Zilla Parishad, Nagpur, has been trained to undertake rapid nutrition survey amongst school children of this C. D. Block.

Hospital Dietetics.—At the request of the Medical Superintendent of M. G. M. Hospital—a quasi-Government Hospital amidst the thick populated industrial area (Parel) of Bombay (admitting E.S.I.S. patients)—a visit was paid to the diet kitchen, ward pantries etc., with a view to explore the possibility of improving its food service in general and the menu meals, in particular.

Routine visits to Government Hospitals were continued.

Community Nutrition :

(A) *Institutional Feeding Centres.*—Study of dietary conditions in residential institutions (hostels), industrial and other canteens, etc., has been a routine activity in the sphere of practical dietetics. Based on prevailing food habits and the special nutritional needs of the beneficiaries concerned, suitable modifications have been suggested from time to time to provide a 'better' diet, if not a 'balanced' diet, within the budget and other limitations of the management.

The important developments thereof are summarised below :—

(i) *Nursery Schools.*—The extent of protein malnutrition amongst pre-school age child population is rather high. On the other hand, facilities for providing proper nourishment to them, through well-planned and resourceful nursery schools, such as the Bal-mandirs or the Balwadis, are rather inadequate, even in the Cities.

In this context the efforts of the Parsi Panchayat Trust in conducting a multi-purpose 'Infant and Nursery School' in the Fort area of Bombay for the last 23 years, is praiseworthy.

For the beneficiaries (about 55 to 60 toddlers) who spend here a major part of the day, the maintenance of their physique and health through a Feeding Programme is given priority. From the study of the existing pattern of serving the menus, it can be stated that while

caloric content of the total food consumption has been observed to be slight inadequate, the average intake of 'protective' foods—mostly received as gifts, viz., skim milk powder, fresh fruits and butter, was adequate.

(ii) *School Children*.—A welfare organisation (name : Ismailia Helping Society) caters to the needs of the poor children attending the primary and secondary school in a Muslim area of Bombay. At the request of the women workers of the Society, the services of a low-cost lunch to the children (about 180 between the ages of 5 and 15 years) was studied. Adequate quantity of rice and dal, along with pieces of meat and potatoes, from the lunch which is prepared hygienically at a place called 'Apni Duccan' and transported to the School premises.

Inclusion of green leafy vegetables, toned milk (about 100 ml.) and half-a-banana (about 40 g.) have been suggested not only to provide balanced nutrition to this growing children but to promote healthy food habits in the formative period of their life. The newer knowledge that a combination of cereals provides better amino-acid pattern to the system, has been applied here by recommending a slice of bread (with a thin spread of butter) to supplement about 90 g. of raw rice.

(iii) *Military Boys' Hostel, Dharwar (Mysore State)*.—With a view to minimize the expenditure incurred on food at the Hostel, mentioned above, a request was made to study the existing diet and furnish a balanced diet for the inmates, attending either a High School or a College at Dharwar in the neighbouring State of Mysore.

Taking into consideration, the nature and extent of their activities in School/College, the inmates would not require more than 2,700 calories; whereas they were actually consuming excessive quantity of starch cereals, providing excess calories. A reduction in the cereals-cum-roots along with an increase in the green leafy vegetables was the main recommendation. To be realistic, an intake of 150 g. on alternate days, suitably combined with *dals* and/or other vegetables, was specially suggested.

(iv) *Home for Adolescent Girls, Dadar, Bombay*.—There is an Institution (Name : Bombay Vigilance Association) in Bombay which offers protection to deserted or otherwise helpless girls and women.

At present food and shelter is given to about 50 inmates, of whom 5 are children (below 15 years) and 43 adolescents. The dietary condition in this Institute was studied during April-May.

Taking into consideration the nature and extent of their activities, the consumption of caloric-rich foods was rather high. Along with their reduction, an increase in the amount of green leafy vegetable, such as 'chowli', 'methi' etc. was recommended in this Institution also. A suitable increase in the inexpensive yet nutritious groundnuts was specifically suggested.

(v) *School for the Blind, Dadar, Bombay.*—In this special school, over 130 blind boys, mostly adolescents reside.

Taking into consideration their age and activities, the food intake was low. In order to rectify this under-caloric diet a slight increase in the cereal-cum-pulses were recommended. On the other hand, the intake of 'protective' foods like skim milk powder and butter—mostly received as gift—was adequate. Provision of more of green leafy vegetables was suggested here also.

(B) '*Square Meal*' Canteen, *Sachivalaya, Bombay.*—This is a Government subsidised canteen, for the benefit of the ministerial and other staff of the Secretariat. It continued to cater a composite, vegetarian lunch consisting of 'chapatis', rice, 'dal', green vegetables, a salad, chutney and buttermilk at 45 P. It provides about 1,000 calories and balanced quantities of good quality proteins etc. This inexpensive and nutritious meal is served to over 900 persons daily. Being a demonstration centre for the planning of mid-day meal at a reasonable price in large non-industrial centres of group feeding, many interested visitors are taken round the canteen. Periodical (once a month) visits are paid by a dietician to assist the management in the maintenance of hygienic food service and the nutritional standard of the meal.

NUTRITION EDUCATION AND PUBLICITY

(A) *Exhibitions.*—Planning of suitable 'poster display' in exhibition sponsored by Government/voluntary organisations, has been continued.

During the period, the Department participated in 8 such exhibitions including one in New Delhi.

Depending upon the main theme of the Exhibition, suitable low-cost nutritious recipes have invariably been displayed on every occasion.

The innumerable visitors—big and small—evinced intelligent interest in the Nutrition Section, and were particularly impressed by the palatability

of a sweetish snack in which the protein-rich oil expelled groundnut (low-fat) flour was used. It is appropriately called VIKAS PAK, for it is particularly suitable for the growing children.

(B) *Experimental Kitchen Work*.—While the information on the nutritive value of individual foods, as purchased, is readily available, there is paucity of information on the caloric and nutrient contents per serving of food combinations as prepared and consumed by the different communities. To obtain this scientific data, the fully qualified lady nutritionists continued the work by actually preparing and standardising about 10 such recipes. Moreover, six low-cost nutritious recipes formulated anew, efficiently tried and found acceptable to the 'grihinis' in the near part, have similarly been standardised. The recipes were palatable combinations using inexpensive yet nutritious plant foods such as radish leaves in *parothas*, *rugi* (Syn. *Nachni*) as sweetish conji, *PALAK* in raita, *GREEN MONGRI* in raita. *Drumstick leaves* in *Meduwada* and *Mung Dal* in *Sago-wada*.

(C) *Nutrition Cookery Demonstrations*.—In view of its utility and popularity, the technique of educating the 'grihinis' by an actual demonstration of any two nutritious, yet palatable, food combinations, from locally available low-cost foods, has been further extended to cover many available low-cost foods and more welfare centres during the year.

In all 35 such demonstrations have been given, covering six welfare centres, spread all over Greater Bombay and one in Ambernath (District Thana).

(D) *Food Competitions*.—Our efforts to popularise low-cost nutritious recipes have evoked favourable response from several organisations. Many of them have been stimulated in recent months to plan nutritious food and cookery competitions amongst their members. The experienced Educationists of the Nutrition Department have been frequently requested to serve on the panel of judges on such occasions.

From our experience of having judged about 1,000 recipes, it can be stated that the modern "grihini"—though not academically educated, is quite nutrition-minded and has grasped the value of 'protective' elements and nutritious cooking in improving her family's diet.

(E) *Popular Talks with Food Display*.—In response to request from social welfare organisations, talks in popular language on subjects of topical interest are arranged, catering to the needs of a broad spectrum of knowledge-hungry individuals and health-conscious groups, such as students of Social Sciences, Social Workers, the middle-class intelligentsia pursuing

various vocations, 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 provides the practical experience to us, so basic to the more essential work of standardising the recipes—as narrated earlier and popularising them amongst a wider and wider circles of nutrition-minded population.

(F) *Orientation in Nutrition*.—Improved techniques with unfamiliar but nutritious food combinations are being evolved by fully-trained women nutritionists here, as part of the State Nutrition Organisation's efforts to achieve a 'better' (if not a balanced) diet for the masses. Proper use of locally available foods in attractive combinations has been the aim and it has been promoted considerably by persuasive education including demonstration in nutritious cooking.

The experience thus gained is utilised in imparting orientation training to rural para-medical personnel and notable progress has been made at the Health Units of Palghar and Sirur.

The response to this simple initiation into practical dietetic work, as judged by the wide variety of acceptable food combinations, rich in proteins and other protective elements, displayed by the nursing staff and students of the Rural Health Units, was very encouraging.

Giving similar guidance, the rural health personnel are capable of computing the dietary habits and subsequently developing an educational programme involving better food habits and proper cooking practices. Once oriented in rural nutrition work, this health personnel can significantly assist the local Health Administration in improving nutritional health, specially of mothers and children, as part of integrated health education. This newer approach of imparting orientation training in nutrition, effectively launched in Maharashtra, is particularly essential in our country, where there is hesitancy on the part of highly qualified nutritionists or social science graduates, to take up educational work in the rural areas.

TRAINING IN NUTRITION

(i) *Medical Internees*.—Soon after passing their final examination, the medical graduates are stationed at one or the other of three Rural Health Training Units of the State. During their stay of 6 weeks or more, these

'internees' are acquainted by the Medical Nutritionist with the techniques of evaluating the food consumption levels and the clinical assessment of the villagers, by actual visits to the farmer's dwelling and the elementary school. Thus, the 'internees' prior to commencing their professional responsibilities are provided with an opportunity to learn the role of diet and nutrition in the causation of ill-health of the rural communities and at the same time, the base-line data regarding the levels of dietary intake and the incidence of malnutrition, is obtained.

Once the average dietary intake for the Unit Area is computed, suitable charts will be locally prepared and displayed in the Health Units Museum, as well as utilised for the forthcoming Training Programme in Applied Nutrition Project with UNICEF Aid.

During 1964 and January, March 1965, all the three Health Units, viz. at Pulghar (near Bombay), Sirur (near Poona) and Saoner (near Nagpur) were effectively covered.

(II) *D. P. H. Students*.—Medical Graduates of Bombay University, who had enrolled for the Diploma Course in Public Health (D. P. H.) were acquainted with the techniques in clinical assessment of nutritional status, particularly of the toddlers and school-age children.

The methods and material for devising nutrition education programme as part of health education, both in urban and rural environment, was specially elaborated. Modern techniques in biochemical and animal experimental research work was narrated to them.

(III) *Bhagini Samaj's Social Worker*.—With the grant-in-aid sanctioned by the Union Ministry of Food and Agriculture, New Delhi, the Health Committee of the Bhagini Samaj, Khetwadi, Bombay, has planned to initiate the 'grihinis' of middle income group of Greater Bombay in elementary nutrition and dietetics through women and other welfare organisations willing to participate. This is to be carried out by lecture-cum-demonstration by a lady social worker. For conducting this work on proper lines, a social worker employee of the Organisation was trained by a Senior Lady Nutritionist. The trainee was acquainted with the basic principles of nutrition and the nutritional merits of commonly consumed food-stuffs vis-a-vis the common problems of malnutrition and ill-health associated with faulty and defective dietary habits, prevalent amongst the middle-income groups of society.

(iv) *Pediatric Nurses*.—The Medical Nutritionist, on being invited, gave a series of 10 lectures to the batch of ten (fully qualified) nurses attending the post-certificate course in Pediatric Nursing, in early 1964-65 at College of Nursing, Bombay. Different aspects of Nutrition and Dietetics as applied to pediatric practices, were covered.

ROUTINE

The total number of tests on clinical materials carried out during the year under review was 2,944. Of these, the special tests numbered 613 shown below :—

Blood	...	...	...	1,988
Urine and Stool	...	...	...	215
C. S. F. and Body Fluids	...	...	...	127
Special Tests	...	...	...	613

In addition to these 11 samples of food material such as milk powder, butter, wheat samples and roots were accepted for chemical tests.

DEPARTMENT OF BLOOD BANK

GENERAL

During the year under report, the activities of the department have extended in a new field viz. installation of the plasma fractionation unit. Other routine activities were continued.

National emergency work.—The work of processing and lyophilising plasma from the blood of voluntary blood donors from the State of Maharashtra continued as in the previous year. However, the amount of blood received from the mobile units of Maharashtra State Blood Bank Scheme was much less than in the previous year and hence the supply of plasma to the Armed Forces was also reduced. Unfortunately, there was a sudden lack of enthusiasm in the general public to donate blood, quite contrary to what was found at the beginning of the national emergency. There is a great need to carry out constant publicity and educational work to keep up the donor consciousness. However, this Institute is handicapped for want of proper publicity staff. When the publicity is carried on, there is a good response.

Publicity work.—At the "Our Heritage" exhibition, a blood bank stall was arranged for carrying out publicity for blood donation and an air-conditioned blood donation room was put up. The blood from voluntary

more was collected on the spot and over 500 donors donated blood. The plasma processed out of this was sent to the Defence Forces.

To facilitate the work of the mobile blood collecting units, a new properly equipped ambulance was constructed. It has been fitted up with refrigerated bottle cooler and an electric generator to run the same.

The Dexivac type plasma drying unit which has been in operation since 1950 was showing major defects by way of leaks in freon line. Hence the whole refrigeration line has been over-hauled and the machine is now working satisfactorily.

Plasma fractionation unit.—During the year, this project, which was taken up in collaboration with Dutch Government, has been implemented as part of IIIrd Five-Year Plan. Two scientists of this Institute who had been deputed to the Central Blood Transfusion Service Laboratory, Amsterdam, returned after finishing their training there. All the items of equipment gifted by the Dutch Government were received during the course of the year. The cold temperature laboratory was also put into commission, though it is not still working quite satisfactorily. The Dutch expert Dr. H. W. Krijnen, Assistant Director, Central Blood Transfusion Laboratory, visited this laboratory to guide the process and was here for two weeks and six batches of plasma have been processed into various fractions e.g. fibrinogen, gammaglobulin, albumin and pasturized plasma for transfusion. Products are under test.

Training of the medical and para-medical personnel.—The personnel deputed for training in this technology by the State Government and other institutes were given facilities for training.

Staff. Shri C. J. Karkhanis, M. Sc., joined this department as Junior Scientific Officer, after finishing his training at the Central Blood Transfusion Laboratory, Amsterdam, in connection with the plasma fractionation project. His training would be valuable for new project.

The shortage of laboratory space is being keenly felt. All the equipment received cannot be installed and worked. The estimates and plans for the new building have been submitted to the Government for administrative approval.

The routine of collection and processing of blood into lyophilised plasma and preparation of diagnostic blood group reagents and carrying out of the blood testing on the samples referred was continued throughout the year.

RESEARCH

1. *To study the nature of contamination of blood during the large-tapping blood donors outdoors.*—A study on 1825 blood donations at different centres viz. at the Institute, at the mobile units in the city at the mobile sessions in the mofussils was carried out. Of these 1,000 without any bacteriostatic agent and 275 each with one of the three bacteriostatic agent tried (sulfathiazole, sulfacetamide, sulfasozole). It was found that there was no significant difference between the rate of contamination observed without the use of the bacteriostatics and with the use of any of the three bacteriostatics.

2. *To standardise methods of production of blood group reagents*
(i) *Anti-human globulin serum (Coomb's reagent).*—The reagent is now produced by using the whole blood serum as antigen using rabbit as animal. This has been done and quite satisfactory reagent has been obtained by following Proom's method.

In order to obtain more specific anti-serum against gamma type anti (like Rh antibodies) a more specific antigen like the gamma globulin fraction may be preferable.

So gamma globulin fraction from human serum prepared locally used. The antigen was prepared by Proom's method and rabbits immunised.

(ii) *Anti-human globulin serum production in larger laboratory animal.* Since the yield of serum from rabbit is rather limited, it would be advised to produce it in larger animal. A sheep was taken up for this purpose. The first course of immunisation did not show satisfactory response. After a period of rest, the second course of immunisation is undertaken.

(iii) *Specificity test.*—The species specific antisera against common species like anti-bovine, anti-equine, anti-canine have been produced and being used to test the products produced in the Department.

3. *Placental blood serum fractionation.*—The placental blood is collected from neighbouring maternity hospital. The serum is separated and stored in deepfreeze till sufficient quantity is collected. This is highly haemolysed. By following the process of chloroform extraction followed in Czechoslovakia it has been possible to get rid of this haemoglobin and then further fractionate the serum.

ROUTINE

The following table shows the routine production from 1st April 1964 to 31st March 1965 :—

(1) No. of Blood Donors—

1. Voluntary	...	...	1,067
2. From prisons	...	...	4,452
3. On payment	...	...	2,416
4. M.S.B.B.S.	...	...	2,377
5. Other hospitals	...	...	1,148
			11,460

(2) Plasma prepared ... 967.5 litres. 3,870 bottles.

(3) Plasma supplied ... 986 litres 3,944 bottles.

(4) Blood grouping sera—

1. Anti-A	..	18074	Ampoules Manufactured	..	18100	Ampoules Supplied
2. Anti-B	..	18303		..	17891	
3. Anti A+B	..	426		..	351	
36803 ampoules				36402 ampoules		

(5) Ampoules of Antivenine ... 61,269
dried.

(6) Rh testing	...	...	Paid	108
	...	...	Free	245
				353

DEPARTMENT OF CHEMOTHERAPY

GENERAL

During the year under review, the progress in all aspects of the Departmental activities was maintained. The research work was continued with the help of research scholars, fellows, post-graduate students and departmental staff. The main fields of problems of study comprised synthesis of antimicrobial agents, possible diuretics, psychotropic substances and cytotoxic agents. The drug screening unit was able to carry out screening of antifungal agents in addition to the screening of antitubercular and other antibacterial testing done previously. Production and development of clinical products also showed improvement over previous year. Plant studies on oral antidiabetic drugs were continued satisfactorily. Eleven post-graduate students including staff and research fellows were in the department. Four science graduates took advanced training in the Technicians' Training Course.

RESEARCH

Antitubercular compounds.—In view of the good *in vitro* antitubercular activity shown by R-47, a thiazolylthiourea derivative synthesised and tested in the department earlier, a further series of compounds related to it and containing halogens in the phenyl ring were taken up for synthesis. Four compounds so far prepared have been submitted for preliminary screening test.

Another series of compounds, viz. 3, 4-disubstituted 5-carboxy-mercapto 1, 2, 4-triazoles have been prepared as possible antitubercular antifungal agents. Out of 25 compounds prepared, 20 have been screened and 8 among these have shown *in vitro* antitubercular action.

2-Pyrazolin-5-ones and 2-isoxazolin-5 ones have been prepared for study of their antimicrobial action. Out of 35 compounds prepared representative compounds were screened for antitubercular activity. Isoxazolin-5-one derivatives showed good antitubercular action (in range of 10 mg./ml.) while in the pyrazolin-5-one the highest activity encountered was at 40 mg./ml.

Diuretic compounds.—A big series of triazine derivatives was synthesised as possible oral diuretics. Out of the 40 representative compounds submitted to the C. S. I. R. Unit working in the Pharmacology Department, Seth G. S. Medical College, 8 compounds have passed the preliminary

screening tests as suitable for further testing. Efforts made to improve the absorption of R-37, by introduction of hydrophilic groupings in the molecule have not been successful. One compound prepared showed better absorption as well as diuretic action in rats (compared to R 37).

Cytotoxic substances.—The work on the Schiff's Bases from Benzaldehyde nitrogen mustards for testing against Dunning Leukemia was continued. Seventeen more compounds were prepared in sufficient quantities and sent to Cancer Chemotherapy National Service Centre, N.I.H., Bethesda (U.S.A.).

Preliminary screening reports of 10 compounds out of 14 sent earlier have been received. These reports give data on toxicity, and effects on Dunning Leukemia, Walker Carcinosarcoma and on K. B. Cell Cultures. In general, the compounds were of low toxicity. In Dunning Leukemia, two compounds showed activity. Mean survival for one of them being 150 per cent. of untreated with 2 cures out of 6. The second showed 140 per cent. mean survival. In Walker Carcinosarcoma, 3 compounds out of 10 showed tumor weight reduction ranging from 39 per cent. to 62 per cent. In view of these test reports, now it is possible to correlate the structure with activity and it is planned to prepare newer derivatives in the light of results obtained in the testing.

Psychotropic and CNS active substances.—Synthesis of *beta*-hydroxyphenylmethyaminoheterocyclic compounds was continued. A large number of intermediates have been prepared and so far 5 final compounds have been prepared.

Fresh supplies of toxic varieties of Harik have been obtained and a good amount of stable active fraction has been collected for further isolation and structure studies.

Drug Screening.—Nearly 75 compounds of the following series synthesised in the department were screened for antimicrobial action :—

1. Pyrazolone derivatives,
2. Thiosemicarbazides,
3. Triazole derivatives,
4. Triazine derivatives,
5. Thiazole derivatives,
6. Thiosemicarbazones.

Among 11 pyrazolones from the group (1) screened against *S. ty*, *S. aureus*; *E. coli* and *M. tuberculosis*, only two compounds showed action (at 200 μ g/ml.) against *S. aureus*. None showed significant action against *S. typhi* or *E. coli*. Seven showed action against *M. tuberculosis* one at 40 μ g/ml., three at 100 μ g./ml. and three at 200 μ g./ml.

Out of 6 thiosemicarbazides, 4 showed action against *S. aureus*. was active against *S. typhi* or *E. coli*. Four showed activity of low order against *M. tuberculosis*.

Out of 11 triazoles, 3 compounds were active against *S. aureus*. was active against *S. typhi* or *E. coli*.

Out of 21 triazoles, 17 compounds showed action on *M. tuberculosis* in the range of 20 to 100 μ g./ml.

Three triazines showed slight activity against *S. aureus* (100 μ g./ml.) only two showed antitubercular action, in the range of 40 to 100 μ g./ml.

Similarly out of 14 thiazole derivatives, 6 showed antitubercular action.

Seven thiosemicarbazides showed no action against *S. typhi* or *E. coli*. Six of them, however, inhibited *M. tuberculosis* at 100 μ g./ml. and on 200 μ g./ml.

In the preliminary antitubercular screening only dilution up to 20 μ g. was the highest dilution used. Compounds showing inhibition at this dilution would be tested at higher dilutions.

Guinea pigs fed on diet containing a potent fraction obtained from *Vakeri* roots showed presence of antitubercular activity in their serum.

Three different strains of *M. tuberculosis* resistant to different antibiotics were obtained from Tuberculosis Laboratory of Pasteur Institute, Paris, and are being maintained. The growth of two of the strains is very much poorer and slower than the usual strain (H_3 , Rv) it has been possible to use them for antitubercular testing for finding out suitable drugs against resistant strains. It is proposed to carry out further investigation for better media for these strains.

ROUTINE

During the year under review, the department continued to manufacture and supply vitamin, sulpha drugs and quinine tablets to the hospitals and dispensaries of the Government and Municipalities and to Employers.

the Insurance Scheme. The table shows the products manufactured during the year :—

Serial No.	Name of the product (Tablets)	Number of Tablets manufactured
1	Vitamin AD (Pediatric)	... 12,518,900
2	Vitamin A Forte	... 154,700
3	Vitamin AD (Coated)	... 1,808,050
4	Vitamin BC	... 27,986,608
5	Vitamin BC No. 3	... 1,369,800
6	Vitamin B ₁	... 2,754,050
7	Vitamin B ₂	... 391,486
8	Vitamin C	... 6,236,918
9	Acid Nicotinic	... 1,006,428
10	Nicotinamide	... 51,250
11	Multivitamin	... 30,166
12	Vitamin K	... 278,440
13	Calcium with Vitamin D	... 1,647,280
14	Magnesium Trisilicate	... 1,259,100
15	Isoniazid	... 3,303,840
16	Isobutamide	... 311,090
17	Sulphathiazole	... 6,879,500
18	Sulphadiazine	... 18,469,000
19	Sulphaguanidine	... 9,418,500
20	Quinine Sulphate	... 321,900
Total		... 126,333,578
1.	Pemon	... 25,433,692 litres.
2.	Sulphathiazole Cream	... 77·180 kg.
3.	Solapsone Strong B. P. C. Solution	... 354 × 50 ml.

The Analytical Section of the department analysed 1,390 samples of raw materials and finished products during the period of April 1964 to March 1965 and 632 micro-analysis of compounds synthesised in the department were carried out.

DEPARTMENT OF CLINICAL PATHOLOGY AND DIAGNOSTIC REAGENTS

GENERAL

The department continued to carry out its varied activities as in previous years.

Six post-graduate students, 9 short-term trainees and a batch of Technician Trainees were given training. Four post-graduate students complete their studies and were conferred the degree of M.Sc. by the Bom University.

The Diagnostic Reagents Section is being reorganised. Some staff been sanctioned. The building construction awaits administrative appro

RESEARCH

1. *Rapid laboratory diagnosis of clinical diphtheria by Fluorescent A body technique.*—So far about 300 samples of diphtheria swabs have investigated by the conventional laboratory methods of culture, toxigenicity by *in vivo* and *in vitro* methods and also by Fluorescent A body Technique. For the latter, both the antitoxic and anti-bacte conjugates were prepared for use. Suitable bacterial and other cont were employed. The results show nearly 95 per cent. agreement betw the two tests. The final diagnosis can be given within few hours of arrival of the swab in a laboratory. Thus once established, this test simple to carry out and also quick in the diagnosis of diphtheria.

2. *The study of epidemiology of typhoid.*—The term 'Epidemiol used here is only in a limited sense and does not cover all the aspects typhoid fever. Only few selected topics have been taken up for det study according to the availability of the material, co-operation and sc of the work. The following problems connected with typhoid have taken up :—

A. *Pattern of agglutinins following TAB Inoculation.*—About 300 have been inoculated amongst the N. C. C. cadets of S. I. E. S. Coll Sion. These are being followed up. Two months after the inocula about 40 per cent. of them did not show any antibody titre and at end of four months, 30 per cent. more were without any titre.

Another group of 120 adults inoculated with TAB Vaccine is u observation for detection of any enteric fever or carrier state in coll ration with Dr. D. N. Pai of Preventive Medicine Department, G.

Medical College and Gujarat Research Centre, Khar. Their base line titre was negligible before inoculation.

II *Comparative study of the antigenic properties of Typhoid Vaccines prepared from freshly locally isolated strains and the standard strains.*—For the six locally isolated strains vaccine has been prepared by two methods, i.e. heat killed phenol preserved and formalin killed and preserved. The standard vaccine from WHO Ty2 strain is also similarly prepared. These vaccines have passed all the tests except the potency for want of enough animals. Meanwhile, possibility is being explored to make use of eggs in place of animals.

III *Comparative studies of different techniques for the early serological diagnosis of typhoid fever.*—This is tried by different techniques like fluorescent antibody detection, Coomb's test and Precipitin Test along with Widal and cultural reports. So far about 100 non-typhoid sera were tested and the base line titre for the normals has been obtained. Specific high titre sera against the enteric group of organisms have been prepared. The conjugate prepared, both for direct and indirect fluorescent antibody testing, is further being purified by column chromatography using DEAF cellulose. Antihuman globulin serum is produced in rabbits. The standardization of Coomb's test is nearly completed. The patients' sera of 52 typhoid cases diagnosed culturally are processed and preserved for testing by the above techniques.

IV *Sugarcane juice bacteriology.*—About 35 samples have been processed so far from the different parts of the city. The following strains were isolated: *E. coli* (typical) 29; *E. coli* (atypical) 8; *B. proteus* 6; and one each of the following types—*S. aureus* (coagulase positive), *Streptococcus* (non haemolytic), *Alkaligenous faecalis*; and yeast cells. After completing 100 samples of these, other similar materials like *Neera*, *Panipuri*, *Bhelpuri*, will be taken in hand.

V *Some biochemical studies on the enteric group of organisms.*—Strains used for TAB Vaccine (*S. typhi* Ty2 and locally isolated paragraph A and B) have been used for this work. So far, nucleic acids (DNA and RNA) have been estimated. Antigenic studies by agar gel diffusion and immunoelectrophoresis are carried out. The various amino acids estimations are in progress.

VI *Comparative Studies of Anonymous Mycobacteria and M. tuberculosis from human and bovine sources.*—This is to find out microbiological

characters of a typical and *Anonymous Mycobacteria* and to compare with *M. tuberculosis*. So far 20 strains have been systematically investigated for their morphological, cultural, biochemical and biological characters various media and *in vivo* studies. This will be useful for their differentiation from the typical ones. The problem is being continued.

6. *Serological phage typing and other studies of pathogenic staphylococci*.—About 200 strains of pathogenic staphylococci have been isolated from this laboratory and other places. Phage typing of all these has been carried out against a basic set of 21 phages. Special immune and absorber 14 rabbit factors sera are prepared for serotyping. In addition to these certain other biological characters are also investigated like sensitivity tests and mercuric chloride resistance test etc. The work is in progress.

7. *Study of gonadotrophic hormonal levels in cases with bad obstetric history*.—Study of cases like repeated abortions, still-births, etc. is made in collaboration with a Research Unit at the K. E. M. Hospital. Quantitative estimation of C. G. H. by immunological and biological methods are made. About 20 cases have been investigated by both methods after standardization of the C. G. H. These cases are being followed up by repeated and periodic estimations of the hormone level.

ROUTINE

1. Smear for T. B.	...	...	...	437
2. Culture for T. B.	...	...	...	88
3. Animal Test for T. B.	...	...	...	34
4. Differential Count	...	...	...	788
5. Total RBC	...	...	...	686
6. Total WBC	...	...	...	734
7. Haemoglobin	...	...	...	584
8. Blood culture for other diseases	...	...	...	27
9. Blood culture for typhoid	...	...	...	1,152
10. Urine culture	...	...	...	265
11. Stool culture	...	...	...	70
12. C. S. F. culture	...	...	...	1
13. Miscellaneous culture	...	...	...	116
14. Autovaccine	...	...	...	48
15. Diphtheriae smear and culture	...	...	...	399
16. Wassermann tests	...	...	...	141
17. Kahn tests	...	...	...	581

18	Widal tests	...	...	...	1,511
19	V. D. R. L. tests	...	...	...	132
20	Pregnancy (Qual.)	...	...	...	2,248
21	Pregnancy (Quan.)	...	...	...	10
22	Nemen	...	...	...	121
23	Stool for cholera	...	...	...	569
24	Urine routine	...	...	...	6
25	Smear for Gonococci	...	...	...	377
26	Stool routine	...	...	...	57
27	Rideal Walker Coefficient	...	...	...	4
28	Blood for M. P.	...	...	...	9
29	Blood for Prothrombin Time	...	...	...	25
30	Throat swab for culture	...	...	...	3
31	Blood for filaria	...	...	...	249
32	Antibiotics sensitivity	...	...	...	Nil
33	Prol's test	...	...	...	69
34	C. S. F. routine	...	...	...	12
35	Ascltic and other fluids cells count	...	...	...	3
36	Leptospira & Cold Agglutination test	...	...	...	4,400
37	Histopathological tests	...	...	...	4
38	Bone marrow smear	...	...	...	1
39	Tuberculin test	...	...	...	2
40	Casoni's test	...	...	...	

None of the interesting specimens received during the year were as follows : —

Dermoid Tumour, Ovarian Fibroma, Gonad (Testicular) Hermaphrodite, Hydatid Cyst, Melanoma of Eye, Urethral Caruncle, Chorio-carcinoma of Uterus, Hodgekin's gland, Teratoma Testis, Infiltrating Duct Carcinoma Brest, Chorio-carcinoma vaginal wall and Thyroglossal Cyst.

Production of Diagnostic Reagents

1.	Dead Emulsion	...	...	...	4,59,455 ml.
2.	High-titre sera and Amboceptor	...	...	...	4,212 ml.
3.	Kahn antigen	...	...	...	18,840 ml.
4.	Wassermann antigen	...	...	...	2,020 ml.
5.	R. V. Venom solution	...	...	...	823 ml.
6.	Cobra Venom solution	...	...	...	753 ml.
7.	Casoni's antigen	...	...	...	587 ml.
8.	Biological stains	...	...	...	7,36,500 ml.

Supply of Diagnostic Reagents

1. Dead Emulsion	...	...	3,67,775 ml.
2. High-titre sera and Amboceptor	...	...	2,488 ml.
3. Khan Antigen	...	...	16,370 ml.
4. Wassermann antigen	...	...	2,700 ml.
5. R. V. Venom solution	...	...	703 ml.
6. Cobra Venom solution	...	...	1,066 ml.
7. Casoni's antigen	...	...	378 ml.
8. Biological stains	...	...	7,72,680 ml.

DEPARTMENT OF ENTOMOLOGY

GENERAL

The Assistant Director attended the quarterly meeting for the co-ordination of plague work in three States of Andhra Pradesh, Mysore and Mad 3rd I. S. I. meeting on transport of live animals committee at Calcutta symposium on pesticides at Bangalore. He was deputed to attend Silver Jubilee Conference of the Entomological Society of India and India Entomological Conference at New Delhi and also the I. C. A. meeting of animal pests of plants. He led a team for the investigation plague at Tiruvannamalai.

Dr. (Smt.) N. S. Rajadhyaksha joined the department as a Pool Offi (C. S. I. R.) and Kum. M. R. Sathe as Research Scholar (ICMR) dur the course of the year.

RESEARCH

1. *Mouth parts of fleas and the feeding mechanism.*—Studies on feeding mechanism of fleas were continued. While feeding, the in pumps the saliva into the tissue of the host. Saliva is believed to mois the tissue of the host thereby making it easy for the insect to pierce skin of the host. An anticoagulant function has been attributed to saliva of certain blood sucking insects. This is necessary for the hae tophagous insects, because the clotting of the blood in the proboscis of insect will hamper the efficiency in feeding.

Saliva secretion of *X. cheopis* has been found to contain a power anticoagulant. The saliva of *X. cheopis* is acidic in nature having a between 5.1 and 5.2. The activity of the salivary gland varies with age and sex of the flea. Homogenates made from the salivary glands

female are found to be more active than those of males. The glands of *Xenopsylla* are larger in females than in males. The role that saliva plays in the process of digestion in fleas is not yet known, but in certain other blood sucking insects the removal of salivary glands does not upset the digestive processes of insects.

The mode of inhibition of the processes of coagulation and the effect of collection of the blood sample of different rats is under study.

3 *Testing of insecticides*.—Studies on the susceptibility of fleas to DDT and other insecticides were further continued. Fleas collected from different localities are being tested for their susceptibility to DDT by exposing them to "WHO" impregnated papers. Fleas tested so far from urban areas of Bombay and nearby rural areas are found to be susceptible to DDT. This work is in progress.

4 *Development of methods to take specific and particular fleas indices in general*.—Work was carried out at Worli Village with plastic bags and later with cover bags. A new technique was tried wherein a thin layer of sand was placed below the wonder traps. A good number of fleas were recovered from the sand indicating the possibility that wandering fleas as well as fleas in transit on the body of the rat might be recovered in this way. Flea index of this locality was high. During the investigation on fipronil at Tiruvannamalai and adjoining district both cover bags and plastic bag techniques were tried and the latter proved helpful for wide scale experimentation.

5 *Studies on M. Sorbens*.—Collection of *M. sorbens* is being done from the Haffkine Institute compound. Having completed the morphological studies, attempts are now being made to breed and rear this fly in the laboratory for work on endocrine glands.

6 *Morphological Studies on rats, fleas and flies*.—The colonies of *Xenopsylla*, *X. astia*, *M. nebulo* and *P. americana* are being reared in the laboratory. The work on the morphology of the two species of rats, viz. *R. rattus* and *B. bengalensis* has been undertaken. The difference in the gut of the two species is being studied. One point of interest in these rats was that the entire gut from gullet to rectum when measured showed that in *R. rattus* the length was 34.5", in *R. norvegicus* it was 38.5", and in *B. bengalensis* 75" and that the caecum in *R. rattus* was 2.5" in *R. norvegicus* 3.5" and in *B. bengalensis* 3.2". It is needed to be seen whether these differences have any effect on their specificity to baits.

6. *The studies on snake salivary glands.*—Experiments to note survival of a snake after removing the salivary gland are being done. Parotid salivary glands from *Natrix piscator*, a non-poisonous snake, removed and the snake is surviving since last six months. Extracts of Parotid salivary glands from different non-poisonous snakes are being tested for common enzymes, by gel diffusion for their toxicity.

7. *Venom output studies.*—Venom output studies from individual snakes in different seasons, sex and locality of collection as well as in snakes from snake farm and snake room are in progress. Venom, when centrifuged, shows different colour tints and leaves much debris in viper venom compared to cobra and krait venom.

8. *Studies on field rats.*—The experiments at Nallasopara were continued. These experiments continued for a period of one year from 28th Feb 1963 in a plain grove touching paddy fields. Ninety-six single rat catch traps were used for these experiments. One hundred and three rats were trapped, out of which, *R. rattus* was most predominant being 64 per cent. of the total catch. Out of the 103 rats marked and released, 21 rats were re-trapped, amounting to about 20 per cent. of total rats released. Twenty-eight fleas were recovered from these rats of which 23 were *X. cheopis*, and 5 were *X. astia*. This data is being statistically scrutinised by the Statistician at I. C. A. R. The experiments were then shifted to a dry tract at Akola on a cotton plantation, on 15th September 1964. Three hundred modified wonder traps with entrances were used along with the single rat catch traps as per the method suggested by Dr. Sukhatme. These experiments were conducted from 15th December 1964 to 24th February 1965. In all 495 rats were trapped during this period. All the rats, except only one (which was *B. bengale*) were found to be *Golunda ellioti*. Out of these 495 rats released, 20 per cent. were re-trapped. Detailed statistical analysis of the data is being done by I. C. A. R.

9. *Susceptibility of R. rattus to Plague.*—*R. rattus* were bred up to several generations by sex hormone treatments. These rats and rats collected from Kurla and Ghatkopar were challenged with a standard dose of *P. pestis*. There was no difference in the immunity status in these laboratory bred young rats and rats collected from Kurla and Ghatkopar. Rats bred in mice of 7th, 8th and 9th generations were found to be highly susceptible to plague.

10 *Rodent control*.—Laboratory trials with anticoagulant rodenticides such as Racumin, Prolin and Warfarin along with other conventional rodenticides like barium carbonate and zinc phosphide were continued. In addition to this, a new rodenticide, Shoxin (McN 1025), supplied by WHO, was also taken up for trial. This rodenticide was tried against three species of common rats, i.e. *R. rattus*, *R. norvegicus* and *B. bengalensis*. The last species of rat did not die in spite of consuming 72 gms. of Shoxin in four days.

11 *Breeding of various species of rats in Laboratory*.—The experiments of breeding rats by artificial insemination, hormone injection and by giving semi-natural habitate in the burrows are in progress. Only the hormone method has so far given two litters. These are being continued to next generation. They have also bred in the burrows.

12 *Collection of mite ectoparasites on rats*.—*R. rattus* and *B. bengalensis* from different wards of the City of Bombay were obtained to get acarine ectoparasites on them. A, B, C, D and F wards of Bombay Municipality have been covered so far. Mites belonging to the families Trombiculidae, Uropodidae, Myobidae, Erythraeidae and Dermanyssidae have been covered so far.

13 *Isolation of fungi parasitic on M. domestica nebulosa*.—The possibility of fungi being employed as a means of controlling insects are being explored in different places. An attempt was initiated on these lines. Three species of fungi *Aspergillus*: sps. *Entomophthora*: sps. *Beauveria*: *bassiana* were used to infect healthy *M. nebulosa* that are being bred in the Laboratory. A mortality of 90 per cent. was seen in the infected houseflies by the 3rd to 6 days, whereas the mortality recorded in the controls was after 11th day. All cultures except *Entomophthora* sps. have been maintained and sent for identification and confirmation.

14 *The studies on the quantitative food consumption by different species of rats and the relation between the quantity of food consumed and the quantity of pellets given*.—The studies on quantitative food consumption by different species of rats and the relation between the quantity of food consumed and the quantity excreted in the form of pellets are undertaken. These studies were carried out with laboratory dry mixed food and carrots. It was observed that the average food consumed per day in case of *R. rattus* was 9.3 gms. in *R. norvegicus* it was 14.5 gms. and *B. bengalensis*, was 14 gms. The percentage of the excreta excreted in the form

of pellets was 9.9 per cent. in *R. rattus*, 7.9 per cent. in the case of *R. Norvegicus* and 12.2 per cent. in the case of *B. bengalensis*. Experiments were conducted to see the time in hours the pellets are given after the meal. In this case the food was coloured with eosin methylene blue. The observations revealed the appearance of coloured pellets after 6 hours of consumption and the colour was observed in up to 48 hours after the consumption of food.

15. *Rearing and Maintenance of colonies of scorpions.*—Colonies of scorpions *Palamneous* sp. *Buthus* sps. are being maintained in the laboratory. The scorpions are fed on laboratory bred cockroaches and of white mice. The venom is collected from scorpions in ampoules lyophilised. These scorpions are kept on a layer of soil spread in a cage. Stone pieces are also placed in the cage with a view to give semi-natural surroundings.

16. *Plague epidemiological studies at Tiruvannamalai.*—In the month of November 1964, a team headed by the Assistant Director was deputed to North Arcot District in Madras state to study the rat and flea incidence in an area with a view to correlate the same to plague epidemiology. These studies indicated that the rat population of North Arcot District consists of *R. rufescens*, *R. rattus wroughtoni*, *B. bengalensis* and *M. musculus*. The last two are very few in numbers and the first one predominates in the area. *R. rattus wroughtoni* were collected particularly in the market areas. *T. indica* were collected from the rat burrows in the hills. *X. astia* was the predominant flea in the locality. *X. cheopis* was found more on the west of Jabali hills at higher altitudes, lower temperatures, in mountainous places and comparatively, more on *R. rattus wroughtoni*. The rat density was high from Tiruvannamalai and onwards to Vellore, but flea indices were insignificantly low except at Krishnagiri where flea indices were high. Flea pools, slides and cultures were found to be negative to plague. Plastic bag technique was tested in the area which gave individual rat indices. Bait box method has been given a trial for reducing flea incidence and as a safe bait station.

ROUTINE

Fifty-two enquiries were attended during the course of the year relating to identification of snakes, insects, rats and insect control. Slides of insects of medical importance, eggs, larvae, pupae of house flies were supplied to various educational institutions. Two bait boxes were sent to Dr. A. M. Francis, Research Officer, Government of Madras.

A single door rat cage was supplied to Director, Virus Research Centre, Pune, as sample. One Cobra Skin was supplied to a local party for its use in medicine.

Training and academic activities.—Four post-graduate students continued their work for Ph.D. Kum. K. M. Karnikar continued as C.S.I.R. Scholar and Mr. R. S. Prasad continued as Government of India Scholar. Plague preventive measures classes were conducted from 1st December 1964 to 19th December 1964. Ten trainees from different public health departments from different States attended the course. Besides this, 16 students of 'Subalterns' Training Course underwent entomological training. Kum. M. Naths was trained in Medical Entomology for three months.

The total quantity of snake venom extracted and supplied during the year was 156.13 gms. and 141.17 gms. respectively. Three thousand four hundred ninety-five snakes were purchased during the year, of which 273 snakes were received as gratis. After a lapse of many years this year again batches of workers from the department were sent to Devgad for purchase of snakes and also for the extraction of venom. The work of producing scorpion venom was resumed this year. Four hundred forty one scorpions were purchased during the year and 15 were collected by the staff members of this department at Deogad. The quantity of scorpion venom extracted and supplied was 0.51 gm. and 0.41 gm. respectively.

DEPARTMENT OF IMMUNOLOGY

GENERAL

The enzyme refined antitetanus sera prepared on a laboratory scale in the previous year were issued for clinical test. The tests showed that enzyme refining has lowered the rate of serum reaction. Small scale production was, therefore, undertaken and about 93 litres of enzyme refined sera have been prepared during the year. Since the last few months, all the anti-plitheria sera have been subjected to enzyme treatment. With the installation of steam jacketed kettle for large scale production of enzyme refined sera, it is hoped to process about 50 per cent. of sera during the next year by enzyme treatment.

The department co-operated in the tetanus prophylactic field trial conducted by Dr. B. J. Vakil at the J. J. Hospital. The trial was jointly sponsored by the department of Technical Co-operation, U.K. and the Maharashtra Government for testing the efficacy of combining active and passive

immunization in patients with tetanus prone injuries. The trial termin at the end of March 1965. About 400 samples of sera have been analy and more are coming in. The results are being tabulated and analysed.

Among the important research work conducted by the department be mentioned the investigations in the "Induction of toxin in diphth bacillus" and on the "lens proteins". Both these are of fundame interest and are likely to have practical applications also. The f research scheme was sponsored for the Haffkine Institute Diamond Jubl Research Unit and the latter for the Dorabji Tata Trust.

The tetanus toxoid marketted during the year was prepared on prot free medium. This was purified and standardized both according to content and antigenic potency. Adsorbed tetanus toxoid which was us the tetanus prophylactic field trials will be marketted next year. Com ed diphtheria and tetanus toxoids in adsorbed form has been prep and has successfully completed laboratory tests. It is hoped to ma this also during the coming year.

RESEARCH

1. *Induction of toxin in C. diphtheriae*.—After the discovery by F man in 1951 that a phage can induce toxin production in a nontoxig strain of diphtheria bacillus, it is believed by workers in this field that ability of the phage to induce toxin production is an inherent and i parable property of the phage. However, we have now been able separate the phage from the toxin producing factor which we have te T+. It is now possible to remove the phage from the bacillus leaving T+ factor intact. Such strains of diphtheria have been found to p more toxin than the phage containing strains. We have also been abl remove the T+ factor from the phages *in vitro* by using small amount acriflavine. Such phages do not induce toxin. They can be mad take up the T+ factor by infecting them into strains of diphtheria w contain T+ and not the phage. The role of phage, therefore, is to duce the toxigenic factor T+ from one strain of diphtheria to another.

These researches have a bearing on the epidemiology of diphtheri the conversion of nontoxigenic and nonvirulent strains into toxino virulent strains with the help of suitable phages containing the T+ fac The work has also shown the possibility of breeding strains in the la tory suitable for producing large amounts of toxin for the preparatio potent diphtheria prophylactics.

1 *Investigation in lens proteins.*—When lenses from eyes are separated and crushed in distilled water and centrifuged, the material separates into two parts, the insoluble fraction and the soluble fraction. The soluble fraction consists mainly of 3 proteins, the alpha, beta and gamma crystallins. The insoluble lens fraction has not been investigated so far because of its insoluble nature. It is however known that the proportion of insoluble lens increases with age and that in a cataractous lens it is about double that of the normal lens of same age.

In our laboratory, the soluble lens fraction was first investigated for organ specific and species specific proteins. It was found that the alpha crystallin was organ specific. This means that it is not present in other organs such as liver, kidney, spleen, brain of the same species but is present in lenses of other species. The beta and gamma crystallins were found to be species specific.

The investigation of the insoluble fraction was then taken up. We have now been able to solubilize it under comparatively mild conditions using 7 molar urea solution. The urea solubilized product remained soluble after removal of urea and it showed an immunological reaction of identity with the alpha crystallin of the soluble part. It resembled soluble alpha-crystallin in other physical properties also, such as electrophoretic mobility and insolubility at pH 5.0. An interesting observation was that the urea solubilized product acquired proteolytic activity like the alpha crystallin though the original insoluble lens did not show such activity. These results indicate that the insoluble lens protein is a polymer of the alpha crystallin.

It has also been possible now to convert alpha crystallin from the soluble fraction into an insoluble protein by the use of alloxan. The insolubilized product can again be converted into soluble alpha crystallin with urea. However, the proteolytic activity is not restored. The action of alloxan seems to be specific since beta and gamma crystallins are not insolubilized.

Preliminary results on the prevention of galactose cataract in rats by 10 percent urea in the diet has shown great promise. It holds some hope that cataract can be prevented.

1 *Isolation of new strains of diphtheria.*—With the background of knowledge of production of toxin by diphtheria, it has now been possible to evolve new strains of diphtheria. A strain has been isolated which produces 40 to 50 Lf. of diphtheria toxin within 3 to 4 days under shaken

conditions. Another variant has been isolated which can utilize sucrose growth and production of toxin instead of maltose, though the orig strain did not utilize sucrose. Use of this strain may make it unn to import costly maltose. Moreover, sucrose can be added directly the medium before sterilization without deferration which is not poss with maltose.

4. *Standardization of pertussis vaccine.*—The following factors invo in the standardization of pertussis vaccine according to N.I.H. method studied : (1) strain of mice, (2) level of testing as compared to stan N.I.H. vaccine, (3) choice of challenge strain.

Results show that among the mice strains tested the Kasauli strain the strain obtained from Glaxo laboratories were the best. The opti level of test is 8 units of N.I.H. reference vaccine per ml. and below. best challenge strain giving repeatable results was the strain 9797. U these conditions the assey was found to be reliable and the results within the expected limits.

5. *Enzymic and non-enzymic degradation of horse antibody protei* It has been found that pepsin digested horse antitoxin can be fu degraded non-enzymatically with sodium sulphate. This product does precipitate with the antigen and retains about 80 per cent. of its anti activity. Work is now in progress to see whether such treatment red the sensitizing activity of enzyme refined horse antisera.

6. *Mode of action of tetanus toxin.*—The mode of action of te toxin is till now not known except that it travels to the brain and ex its action from there. It has now been found in our laboratories that tetanus toxin effects the fixation of energy in the form of A.T.P. in brain mitochondria of susceptible species such as rat but not in the in ceptible species such as pigeon. Further, it has been found that the a leakage of muscle aldolase into the serum during tetanus.

ROUTINE

				April 1964 to March 1965 (Litres)
1. Tetanus toxin—				
(a) Peptic digest medium	...	...	470	
(b) Protein-free medium	...	...	740	
2. Tentanus toxoid—				
Protein-free medium	...	...	727	

			April 1964 to March 1965 (Litres)
1	Diphtheria toxin—		
	(a) Papain digest medium		159
	(b) Protein-free medium		190
1	Diphtheria toxoid—		
	(a) Papain digest medium		101
	(b) Protein-free medium		190
1	Gas Gangrene (Welchii) toxin		106
6	Portusula vaccine		52
1	Triple antigen		70
2	Adsorbed tetanus toxoid (P. T. A. P.) (for field trial).		20

The following quantities of sera were produced during the period :—

1	Tetanus antisera		1,103.4
2	Diphtheria antisera		198.5
1	Anti gas-gangrene sera		124.7
4	Antivenine		705.4
1	Enzyme digested diphtheria antisera		80.8
6	Enzyme digested tetanus antisera		13.0

DEPARTMENT OF PHARMACOLOGY

GENERAL

The nature of the activities of the Department may be broadly divided into 4 groups : (i) Research, both fundamental and applied, (ii) Testing of drugs, (iii) Training of personnel and (iv) Preparing students for post-graduate degrees. Some important features in these fields are summarised below.

Dr. N. K. Dutta, Assistant Director, In-charge of the Department attended the meeting of W.H.O. Expert Committee on Cholera Research at Manila in November 1964. Dr. Dutta also participated in the Symposium on Cholera Research organised by the East West University of Hawaii, held at Honolulu in January 1965. Shri V. Y. More of this Department returned from Amsterdam after completing his training in processing of blood and its substitutes.

Dr. Dutta has been awarded the Doctorate of Science Degree of the Oxford University in December 1964 for his distinguished contribution in the field of experimental medicine.

Dr. N. B. Oza was awarded the Doctorate of Philosophy of the Bombay University on his work on "Studies on *Vibrio Cholerae* and the biochemical and pharmacological aspects of its toxin".

Two Research Scholars obtained M.Sc. Degrees. Kum. Usha Ka obtained her post-graduate degree in Biochemistry of Bombay University for her work on "Studies on oral synthetic antidiabetic drugs". Shri Anil Seth was awarded M.Sc. degree in Biochemistry of Bombay University for his work on "Studies on the nutritional requirements of indigenous lactic acid bacteria".

During the year, the Department lost the services of two experienced workers, Shri K. G. Ananthanarayanan and Shri R. G. Tamhane who were in charge of Biological Section and Pharmaceutical Section of the Department respectively. They were transferred to the newly created Analytical Control Section of the Institute. Dr. N. B. Oza, Ph.D., resigned from post of S.S.O. Class II and proceeded to U.S.A. for further study.

The following people from Pharmaceutical Industry were admitted for short-term training course in the Department. They were (i) Shri P. Shukla, Mercury Pharmaceuticals, Baroda, (ii) Shri N. S. Dhola, Bhalchandra Laboratories, Bombay, (iii) Shri K. Arunajadai, Bahadur Pharmaceuticals, Kumbhakonam and (iv) Shri K. Panchpakesa, Citadel Pharmaceutical, Madras.

RESEARCH

1. *Stability of folic acid in pharmaceutical preparations.*—Folic acid was found to be quite stable in aqueous solutions at pH 5.0 to 8.0. But vehicles like syrup, glycerin, propylene glycol, alcohol and Tween 80 retain most of their folic acid content within the pH range of 6.0 to 8.0. Thiamine, riboflavin, choline and ascorbic acid among the organic compounds and manganese sulphate and calcium hypophosphite among inorganic compounds were found to be responsible for instability of folic acid in pharmaceutical products.

2. *Studies on the stability of vitamins: folic acid and thiamine hydrochloride.*—Studies on the stability of folic acid and thiamine hydrochloride at pH 5.0 and 7.0 and at a temperature of 45°C have been continued. Under the conditions of experiment thiamine hydrochloride did not appreciably effect the stability of folic acid.

3 *Studies on oral synthetic antidiabetic drugs.*—The pharmacological effect of N-benzene sulphonyl N'-isopropylurea (R-94), N-benzene sulphonyl N'-n-butylurea (R-131), N-benzene sulphonyl N'-cyclohexylurea (R-277) was studied and compared with those of tolbutamide, chlorpropamide and DBI. R-277 showed a slight effect similar to the effect of histamine on smooth muscle and R-277 and R-131 exhibited slight analgesic activity and potentiated the effect of pentobarbitone sodium. The experiment showed that R-94 was much superior to the other compounds tested and also to tolbutamide, chlorpropamide and DBI.

The study on enzyme phosphatase showed that phosphatase activity did not seem to run parallel in lowering of blood sugar level.

The compounds R-94, R-131, tolbutamide, chlorpropamide and DBI showed depressing effect on adrenaline induced hyperglycemia except R-277 which had a potentiating effect.

Out of the 10 new compounds tested for their hypoglycemic activity, only one compound R-643 (p-toluene sulphonamide) was found to decrease the blood sugar level in rabbits, rats and guineapigs.

Manganese chloride failed to produce any potentiating effect of tolbutamide in rabbits and guineapigs when fed orally.

4 *Nutritional requirements of micro-organisms in relation to vitamin assay.* Work on the vitamin, amino acid and purine and pyrimidine base requirements of about 10 lactic acid organisms isolated from local sources has shown that all of the lactobacilli, studied required arginine, cystine, glutamic acid, isoleucine, leucine, phenylalanine, tryptophan and valine. Some strains only required alanine and glycine. Lysine was essential for three strains and methionine for one strain. Proline and serine are required by only three of twelve lactobacilli studied. Threonine was essential for only a few strains studied, whilst tyrosine was essential for only one strain.

Glutamic acid, pantothenic acid and biotin were essential vitamins for all the strains studied.

Folic acid, B2 and B1 were required by only one strain. None of the strains require histidine, hydroxyproline, norleucine, PABA, Vit. B6, Vit. B12 and purine and pyrimidine.

5 *Antifertility activity of seeds of *Abrus precatorius* (Linn) on rats and mice.* Investigation was undertaken on the seeds of *Abrus precatorius* with a view to evaluate its antifertility properties, as it has been reported

that they are used for criminal purposes to induce abortion as well as to prevent conception.

The powdered seeds were extracted with petroleum ether and two fractions, viz., oily and crystalline substances were isolated. The oily fraction was able to induce sterility in 60 per cent of the white rats when injected subcutaneously. On oral feeding of the oil varying degrees of sterility were produced depending upon the dosage and frequency of administration in mice and rats.

6. *Recent trend on the pathogenesis of cholera.*—Review of recent experimental work on cholera has indicated that the vibrios after entering the small intestine multiply and undergo lysis as a result of which choleraogenic material(s) is released. There are also evidences which suggest that the choleraogenic component of vibrio lysate (toxins) could be secreted by the vibrio cells. The choleraogenic component of the vibriolysate is non-toxic to white mice, thermolabile and produces watery diarrhoea when administered orally to infant rabbits. In these animals, diarrhoea is produced without any serious damage to the intestinal mucosa both by infection of *V. cholerae* or feeding them with cell-free lysate. In the host there appears to be a competition between lysate production and its degradation by intestinal enzyme (lipase or phosphorylase). When the lysate level gains the upper-hand, it acts locally as well as systemically, the results being the manifestation of the signs and symptoms. Vibriolysate on being absorbed in the body releases histamine from cells, thereby adding to the shock-like symptoms which result from loss of fluid. Vibriolysate has an adverse effect on the heart too.

7. *Morphologic alterations of the intestine of ten-day-old rabbits given intact and ultrasonically disrupted cholera vibrios or cholera endotoxin.* Peroral infection of infant rabbits with *Vibrio cholerae* is a convenient model to study the pathophysiology of this disease. Morphologic alterations of the gastrointestinal tract of ten-day-old rabbits given *V. cholerae* intraintestinally were studied, ultrasonically disrupted cholera vibrios and cholera endotoxin per os were studied sequentially. Animals given 1 vibrio/100 grams body weight developed marked diarrhoea, accumulated fluid in the large bowel and showed diffuse villous and submucosal hyperemia and oedema with focal inflammatory response, and died within 24-hours. Animals receiving cell-free ultrasonicated vibrios filtrate developed diarrhoea, succumbed earlier and had marked villous atrophy.

submucosal hypermia and oedema. Cholera endotoxin per os killed within 24-36 hours without diarrhoea, and produced mucous discharge, accumulation of cellular debris, epithelial cell degeneration and a predominantly mononuclear cell response. Death occurred in all three groups despite an automatically intact intestinal epithelium.

8. *Prophylaxis and treatment of cholera based on experimental evidences.*—In ten-day-old rabbits the intra-intestinal inoculation of small dose of potent *V. cholerae* brings about signs and symptoms resembling human cholera. The animals invariably die with profuse watery diarrhoea and dehydration. The treatment with drugs in the infected animals was arbitrarily divided into 4 categories. The treatment was begun 1 hour before the induced infection or 8, 16 or 24 hours following it. The prophylactic and definite curative values of drugs were judged by their ability to save the animals when the treatment had begun 1 hour before the infection and 24 hours after inoculation respectively. A nine-dose treatment, one dose every 6 to 8 hours was instituted for evaluating each drug. Vibriocidal drugs, e.g., chloramphenicol, chlortetracycline, oxytetracycline, neomycin, novobiocin paromomycin and polymyxin B possessed excellent prophylactic value but were of no use in advanced cases of rabbit cholera. The study further indicated that these drugs would be of some value in early cases of clinical cholera. Likewise, choleraphages prevented the diseases but were ineffective in curing rabbit cholera. Sulphaguanidine and alkaloids of *Berberis aristata* and *Bragantia wallachii*, prevented the onset of experimental cholera and proved to be useful if the animals were treated early. Therefore, in clinical cases effort should be directed towards liquidating the factors which cause choleraic symptoms.

9. *Paramomycin, polymyxin B and furazolidone in experimental cholera.*—Paramomycin, polymyxin B and furazolidone were tested for their ability to inhibit the growth of *V. cholerae* and their effectiveness in curing rabbit cholera. All the three drugs possess powerful vibriostatic and vibriocidal action. Paramomycin and furazolidone could cure about 50 per cent of the animals suffering from cholera if the treatment was instituted early. Polymyxin B on the other hand possesses only preventive but no curative value.

10. *Passive immunity in experimental cholera.*—The infant rabbit technique has been used for the study of cholera vaccines in a passive immunization test, and the results have been compared with the mouse protection test.

Anti-O, anti-OH, vibrio lysate, and live vibrio rabbit (adult) anti-sera protected mice to a varying degree against the challenge of vibrio or vibrio lysate. None of the infant rabbits, however, could be saved from choleraic disease by any of the anti-sera except the anti-live vibrio ones. This remarkable property of the live vibrio anti-sera suggests that vaccine prepared from live culture would be of definite value in humans as an immunizing agent against cholera.

ROUTINE

The Department tested drugs for the Drugs Control Administration for the States of Maharashtra and Gujarat, for Police Department, Prohibition and Excise Department of the Maharashtra Government, Government Medical Stores, Pharmaceutical Industry etc.

Due to the extension of the scope of the Drugs Act to insecticide and cosmetics and the expansion of the Drugs Control Administration of our State, additional burden was thrown on the Department. The staff therefore, could focus less attention to research work and to development of methods. The complex nature of samples gave many day-to-day problems for the development of new methods to test them.

During the year under review, in all 3,691 samples were tested and analysed. These were pharmacopoeial and non-pharmacopoeial preparation and included fine chemicals, insecticides, disinfectants, medicinal toilet preparations, antibiotics, hormones, vaccines, sera etc. Testing of vaccines and sera was done to a limited extent as adequate facilities were not available.

The number of samples that were reported to be of non-standard quality were about 15 per cent. There was a very marked drop in the quality of non-standard drugs as compared to the last year's figure which was about 28 per cent.

Out of the 2,197 samples examined under the Drugs Act, only about 14.0 per cent were of non-standard quality.

The samples that were examined from Police and Prohibition and Excise Departments, amounted to 675. Commercial samples constituted a total of 381.

Biological Section.—Pharmaceutical firms continued to take advantage of the facilities of biological testing that were offered here. But owing to the shortage of laboratory animals, space and preoccupation of the staff

with routine work, facilities for testing of outside samples had to be limited.

About 450 samples were scrutinized by this Section during the year. One hundred thirty-two of them were tested for toxicity. Out of one hundred and fifty samples examined for "pyrogens", 24 samples were reported pyrogenic. Most of these non-standard samples were of "Water for Injection". It appears that still a good percentage of this type of injectable fluids are not free from injurious materials.

Preparation like "Shampoo" was tested for its "Irritant" effect and liver extracts for the presence of histamine-like substances.

Chemical Section.—A large variety of samples was tested and analysed during the year. About 36 per cent of the disinfectants and insecticides that were tested failed to comply with the required standard. It was noticed that some of them had hardly any active ingredients like D.D.T. and B.H.C. which were claimed to be present but contained kerosene, colouring matter and kaolin. Some *Ayurvedic* remedies were found to contain drugs like salicylates, menthol, quinine, sulphur, etc.

A popular remedy known as *Lalpari* was found to contain "Secobarbital". About 35 per cent of the essential oils, pain balms and similar preparations were found to be not of standard quality. About 10 samples of gripe water were tested and reported to contain small quantities of bromides. Another category of drugs, the bulk of which was of sub-standard quality, was tinctures. Forty-one per cent of these alcoholic preparations received from the Police and Prohibition and Excise Department were reported to be sub-standard quality and 19 per cent from these were only coloured alcohol.

Biochemical Section.—Vitamin and antibiotic preparations were extensively tested. Microbiological methods of testing were employed for most of them. Previously (a few years back) the percentage of samples was large. The substantial improvement in the quality is the result of two things. The manufacturers are adding extra amount of vitamins to counteract the loss of potency due to ageing and secondly, incompatible vitamins are being separately dispensed.

It was pointed out before that certain enzyme preparations like pepsin deteriorated under the condition in which they were dispensed. As much as 25 per cent of the preparations containing pepsin, either alone or in

combination, have failed to pass the requisite standards because of the loss of proteolytic activity. More attention is called for to standardise such enzyme preparations. They were in frequent complaints in respect of some injectables for causing some untoward reactions. In most of the cases, it could not be attributed to the quality of the drug. Only in one case it did not comply with the test for toxicity.

Bacteriological Tests.—Seventy-eight samples of bacteriological and serological nature were tested. The samples consisted of anti-toxins, toxoids, vaccines, etc. Most of them were tested for freedom from abnormal toxicity and sterility.

DEPARTMENT OF VIROLOGY

GENERAL

The K. F. D. inquiry is being closed down. The K. F. D. block will be used for virological tests on oral polio vaccine. Dr. K. H. Dave returned from his W.H.O. training in U. K., U. S. A., U. S. S. R. on oral polio vaccine. The polio vaccine building is now under construction.

RESEARCH

1. *Influenza.*—Monovalent influenza vaccines were prepared against four different strains viz. A., A prime, A2 and B. for experimental work. One hundred ml. of polyvalent vaccine (mixture), was prepared for experimental studies. Some interesting results showing overall protection by mixtures of only 2 or 3 strains in high dosage were obtained and will be published in due course.

2. *Vaccinia.*—Hyperimmune rabbit serum against a tissue culture antigen was prepared and standardized.

(ii) The neutralization test in ovo using the two rabbit sera (against rabbit testes antigen and tissue culture antigen) was standardized. Work on the in vitro (tissue culture) neutralization test is progressing.

(iii) The complement fixation test using the anti-sera and vaccinia virus from different sources is standardized.

(iv) (a) Eighty adult human volunteers were vaccinated with Belgaum vaccine lymph. Pre and post immunization (25-30 days) blood samples were collected for study.

(b) 12 individuals, of varying age, were given primary vaccination and pre and post immunization blood samples collected for study.

2. *Studies on Rabies Virus*—(i) *Nutritional requirements of Rabies virus in tissue culture*.—Attempts were made to cultivate rabies fixed virus in cultures maintained in two synthetic media, Eagles' basal medium and 199, and Earles' salt solution with the ingredients of these media individually. The preliminary experiments showed a poor yield of rabies virus as compared to cultures maintained in M+Hanks with serum.

(ii) *Antigenicity of Rabies virus*.—It was observed that harvesting of fluid after prolonged incubation periods at 37°C did not yield more infective virus than when harvests were made at 24 hours (in the secondary cycle of infection). On the basis that the constancy of virus titre indicates a balance between virus replication and virus inactivation, it is possible that inactivated virus present in the fluid may be antigenic though not infective. Carbolized (killed) vaccines were prepared from fluids harvested after varying incubation times and were tested for potency tests. Within limits, the vaccines prepared after longer incubation time showed superior protection.

(iii) *Purification of Rabies virus*.—Studies on purification were continued using tissue culture fluid. Preliminary centrifugation was done at 78,000 g. for 1 hour. The sediment was resuspended and aliquots taken for titration and chemical analysis and also for further treatment with methanol and saturated ammonium sulphate. The final products showed a substantial fall of protein content but also showed considerable loss of titre. It is also of interest that no DNA could be detected in the final fractions obtained by purification with methanol or ammonium sulphate.

(iv) Clinical trials of Hyperimmune Rabies Serum are continuing. 36 other cases have been collected for the series, and follow-up of the cases previous years is continuing.

3. *K. F. D. vaccine*.—(i) *Safety test*.—Safety tests on K.F.D. vaccine were extended to monkeys. No virus could be isolated from 200 ml. of vaccine Batch TC VI inoculated in 12 monkeys by the intraperitoneal route. This vaccine which had already passed safety tests in mice, was, therefore, selected for human volunteers.

(ii) *Volunteer study*.—Two different schedules of vaccination were tried. In the first schedule 48 volunteers each received two doses of 1 ml. 7 days apart and bleeding was done on the 21st day. In the second schedule, the

Second dose was given 1 month after the first and blood samples were taken 1 month after the last dose.

By neutralization tests it was noticed that even though the total amount of vaccine given was the same, the proportion of people in the second schedule responding to the vaccine was higher than in the first schedule. This might be due to better booster effect of the second dose in the 2nd schedule.

4. *Preparation of antisera against Enterovirus.*—Antisera were prepared in rabbits against 3 Polio, 7 Cocksakie A, 4 Cocksackie B and 1 ECHO virus types. The homologous titres of the antisera tested are given in the table. The results of the titration Cocksakie A6 antisera are awaited.

Virus type				Homologous titre
Polio — 1	...	...	...	1,280
Polio — 2	...	...	...	2,560
Polio — 3	...	...	...	1,280
Cocksackie A ₁	...	...	...	16,790
Cocksackie A ₂	...	...	...	14,490
Cocksackie A ₃	...	...	...	3,260
Cocksackie A ₄	...	...	...	8,090
Cocksackie A ₅	...	...	...	28,960
Cocksackie B ₁	...	...	...	500
Cocksackie B ₂	...	...	...	1,990
Cocksackie B ₃	...	...	...	500
Cocksackie B ₄	...	...	...	1,410
ECHO 2	...	...	...	1,410

5. *Poliomyelitis.*—During the period April to July 1964 faecal samples were collected from 188 suspected cases of poliomyelitis admitted to the B. J., Kasturba and Jerbai Wadia Hospital. 111 positive virus isolation were made in monkey kidney tissue culture as follows :—

Virus type				No. of cases
Polio	I	...	...	85
Polio	II	...	...	4
Polio	III	...	...	13
ECHO	9	...	...	1
ECHO	12	...	...	1
ECHO	14	...	...	2
Unidentified	...	...	...	5

(In 8 cases along with polio a non-polio virus was also isolated.)

ROUTINE

(i) *Rabies diagnosis*.—Twenty-two (22) brains of animals (14 dogs, 2 human, 2 cats and one each from female calf, female buffalo, bat and horse) were received for diagnosis. The results of the tests are as follows :—

	Smear	Biological test	Section
Negative	... 13	13	5
Positive	... 3	3	0
Not done	... 6	6	17
Total	... 22	22	22

(ii) During the year several other materials were received for investigation or diagnosis. These included a specimen of lyophilized vaccine lymph for potency, samples of blood and skin scrapings for diagnosis of haemorrhagic small-pox (12 samples) conjunctival smear for trachoma (1) and pus samples for LGV (2).

(iii) Preparation of antigens and antisera for routine and experimental work. Potency and other control tests for the vaccines prepared by the department were undertaken.

Name of the vaccine	Production
<i>Antirabic vaccine—</i>	
(1) Semple's vaccine	... 5,243,253 ml.
(2) Canine vaccine	... 56,250 ml. (9,375 amp.).
(3) No. of sheep used	... 4,605.
(4) No. of centres	... 347 (4 new).
<i>Influenza vaccine</i>	200 ml.
<i>Frei antigen</i>	... 60 ml.

ANIMAL FARM

New animal house has been occupied. Water-supply is now arranged. Some additional staff is sanctioned. Airconditioning could not be undertaken so far.

With a view to obtain a better strain of mice suitable for cholera vaccine testing, a number of strains of mice available in India were brought from various laboratories and tried. But no desirable results were obtained.

A strain of mice was imported from NIH, U. S. A., for the same purpose. Salmonella infection was suspected in these mice also and hence they were all individually screened for salmonella by faecal culture technique which delayed the breeding of these animals to get sufficient numbers for the necessary testings.

Due to the sudden increase in demands from the Bacteriology Department, 235 guineapigs were purchased from other breeding centres for immediate use. At the same time the animals produced by our animal house were reserved for breeding to step up the colony in order to meet the demands in the future. At present, the colony strength has come up to 1352 from 698.

Suddenly, there was shortage of food for animals. This was later arranged for and in some cases substitutes had to be used.

The students in the Technicians' Training Course were trained in the breeding and general management of laboratory animals. Apart from this, the following personnel from different institutions were also trained at this Animal Farm :—

- | | | |
|------------------------|-----|---|
| (1) Shri S. R. Kambli | ... | Wilson College, Bombay. |
| (2) Shri D. K. Ganguli | ... | Indian Institute of Biochemistry and
Medical Research, Calcutta. |

The Table shown below gives the breeding and supply of various laboratory animals from this animal house during this year :—

Animals	No. of animals produced	No. of animals issued	No. of animals supplied	No. of animals purchased
White mice	1,36,909	81,696	704	2,932
White rats	9,372	4,600	4,533	Nil
Rabbits	3,023	2,173	899	Nil
Guineapigs	3,458	3,767	191	235
Hamsters	1,043	171	284	Nil
Cotton rats	90	Nil	Nil	Nil

Besides these, the belowmentioned species of animals were maintained and issued to various departments for experimental purposes whenever required :—

Sheep	...	4,937.
Fowls	...	25 average per month.
Monkeys	...	40 average per month.
Cats	...	10 average per month.
Horses	...	140 average per month.

MAINTENANCE ENGINEERING SECTION

This year both the Desivac and A. E. C. Plant were constantly used. The old plant was completely overhauled. During the year the number of charges were 66. This section is also maintaining cold rooms, centrifuge machine, deep-freeze, ovens, vacuum pumps, air compressors, air conditioners, refrigerators, etc.

The carpenter shop continued to prepare the varieties of crates and small furniture for all the departments. Plants and machinery which have been imported and purchased locally were tested, installed and put to commission.

During the year the Engineering Workshop and Carpentry have been shifted to the Workshop Building and some extra staff added.

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 participated in the Inter-Library loan system, which is in vogue in other neighbouring libraries. It participates in exchange programme under American Medical Library Association, British Medical Library Association and UNESCO. The Library continued to issue the weekly Library Bulletin listing important papers of the journals received week by week.

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 ... 754

B. Loan of Books and Periodicals—

(1) No. of books and periodicals issued to our staff	...	847
(2) No. of books and periodicals issued under Inter-Library loan to other libraries		466
(3) No. of books and periodicals obtained for our staff from other libraries		64

C. Additions of library material during the year—

(1) Total number of books (Accn. Nos. 10356 to 10583)	...	127
(2) Total number of bound volumes of periodicals (Accn. Nos. 8159 to 8614)		455
(3) Total number of current periodicals subscribed	...	101
(4) Total number of current periodicals in exchange	...	200
(5) Total number of current periodicals as gratis	...	107
(6) Total number of periodicals in the current list	...	408
(7) Total number of reports of scientific Institutions received in the library		90

LIST OF RESEARCH PAPERS PUBLISHED FROM HAFFKINE
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- Dutta, N. K. : Recent Trends on the Pathogenesis of Cholera.
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Indian Journal of Medical Science. Vol. 18, No. 3, 1964.
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Indian Journal of Chemistry. Vol. 2, No. 5, pp. 196-199, 1964.

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