



सत्यमेव जयते

1960
1961



ANNUAL REPORT HAFFKINE INSTITUTE

GOVERNMENT OF MAHARASHTRA

CONTENTS

PAGE

1. General Review	1
2. Department of Bacteriology	3
3. Department of Biochemistry	4
4. Department of Chemotherapy	7
5. Department of Clinical Pathology and Diagnostic Reagents	13
6. Department of Entomology	17
7. Department of Immunology	21
8. Department of Nutrition	25
9. Department of Pharmacology	29
10. Department of Virology	37
11. Blood Bank Section	41
12. Media Section	43
13. Animal House	44
14. Maintenance Engineering Section	45
15. Library	45
16. Academic Courses and Training of Health Personnel	47
17. Grants	50
18. Critics Club	52
19. Business Management	56
20. List of Distinguished Visitors	59
21. Miscellaneous—	
(a) Exhibition Activities	60
(b) Haffkine Institute Co-operative Credit Society Ltd.	61
(c) Welfare Activities	61
(d) Supervisory Training Course	62
22. List of Papers published	63
23. List of Papers submitted and waiting for Publication	65

THE HAFFKINE INSTITUTE, BOMBAY

MEMBERS OF THE STAFF

(As on 31st December 1960)

Director

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

Assistant Directors

Dr. A. K. Hazra, M.B.B.S. (Bom.), D.T.M. (Cal.), Assistant Director, in-charge Department of Immunology.

Dr. K. Ganapathi, D.Sc., F.N.I., F.A.Sc., Assistant Director, in-charge Administration.

Dr. N. K. Dutta, M.B. (Cal.), D.Phil. (Oxon.), Assistant Director, in-charge Department of Pharmacology.

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

Dr. M. V. Sant, M.D. (Bom.), F.C.P.S. (Bom.), Assistant Director, in-charge Department of Clinical Pathology and Diagnostic Reagents.

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

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

Assistant Director, in-charge Department of Bacteriology (Post vacant), charge held by Dr. H. I. Jhala, M.D. (Bom.), F.C.P.S. (Bom.), D.T.M. (Cal.)

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

Heads of Sections

Dr. S. S. Sabnis, M.B.B.S. (Bom.), Medical Officer, Blood Bank Section.

Dr. A. E. Kulkarni, G.B.V.C. (Bom.), Senior Veterinary Surgeon, Animal Laboratory.

Administrative and Auxilliary Sections

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

Shri B. O. Shah, B.Com. (Bom.), Cost Accounts Officer.

Shri A. T. Maniar, B.Sc., Stores Officer from 1st December 1960.

Shri Shankar Rao, Personal Assistant to the Director.

Shri T. V. Ranganathan, B.A. (Madras), Office Superintendent.

Shri A. R. Dhavlikar, Accounts Superintendent.

Shri N. Laxminarayana, L.M.E. (Mysore), L.E.E. (Mysore), Maintenance Engineer.

Library

Shri K. N. Pai, B.Sc. (Bom.), B.T. (Bom.), S.T.C. (Bom.), Dip. Lib. (Bom.).
Librarian.

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1960

1. GENERAL REVIEW

The year 1960 is the Birth Centenary year of our Founder-Director Dr. Waldemar Mordecai Haffkine. Glowing tributes were paid to this great personality at a meeting held on 16th March 1960 to celebrate this function. Prof. M. S. Thacker, Director-General, Council of Scientific and Industrial Research was the Chief Guest and Shri M. S. Kannamwar, Minister for Health, Government of Maharashtra presided. The Institute was fortunate to have the presence of Dr. V. R. Khanolkar, Major General S. S. Sokhey and Dr. N. N. Kailas.

This year also marks the birth of the new State of Maharashtra which was celebrated on the 1st May 1960. It was fortunate that the bifurcation did not affect the Institute in any way and the Institute has remained intact so as to be able to serve the areas of the former Bombay State for a period of 5 years as provided in the Bombay Reorganisation Act, 1960.

The State Medical Research Fund, constituted earlier, could not come into existence and was dissolved. The Haffkine Institute Research and Development Fund Committee held three meetings and two meetings of its Executive Committee were also held. The Committee granted sanction to 7 items of construction on the Institute premises at an aggregate cost of Rs. 10,62,919 and 3 items of construction at Pimpri at a cost of Rs. 4,73,800 from resources of the Research and Development Fund. The above works are in progress. Additional 45 acres of land have been acquired at Pimpri. The continued expansion of supply activity in various departments was causing considerable congestion and also encroachment on other activities of the Institute. The problem of space that was seriously confronting the Institute is now likely to be eased a little so as to enable the Institute to prepare for programme of the 3rd Five-Year Plan. From amongst works sanctioned, the raising of storey on the Chemotherapy Wing has considerably progressed.

The Government of Maharashtra has agreed to create three Endowments from the proceeds of the Diamond Jubilee as per the request made by the Haffkine Institute Diamond Jubilee Celebrations Committee. The three endowments are for hostel building, for institution of scholarships and for establishing of a Research Unit, to be manned by 6 junior and 6 senior research fellows.

Dr. K. V. Kulkarni who was on deputation abroad has returned on completion of his training. Dr. J. G. Bhatt obtained his doctorate degree in Pharmacology, while on study leave and has rejoined the Institute. Shri Y. S. Nimbkar has completed his Diploma in Bacteriology from Manchester but continues to be under training abroad.

Dr. Peter Reeves of the Wright Fleming Institute of Microbiology, London, completed his Guest Worker Programme on Bacterial Genetics. Capt. Dan C. Cavanaugh of the Walter Reed Army Institute of the U. S. Army joined the Institute as a Guest Worker to work on the problem of Epidemiology of Plague in the month of March and completed his programme in October 1960. At the instance of Commerce and Industry Ministry of Government of India, the facilities for work were afforded to Madam Zverva from All Union Institute of Experimental Endocrinology, Moscow, for compiling data regarding hormone production from locally available material.

The Yellow Fever Inoculation Centre has been transferred to the Port Health Organization on their premises. A plan for production of oral polio vaccine was drawn and is under consideration. Production of scorpion venom has been undertaken during the year. Mention may be made of the establishment of fluorescent antibody technique for immunological investigations and of development of new method for purification of antisera by phenol.

The building of the Snake and Frog House was brought into commission during the current year. Government have sanctioned construction of additional surface water reservoir so as to provide for a long-felt need for our work. Security arrangements have been strengthened by creating appropriate barricades and walls and extending them wherever necessary. Shortage of adequate cold storage space is holding up the ushering of a programme for planned production. The space in the storage godowns continues to be short. Various short-term, long-term and degree courses continued as usual.

The Section of Biochemistry was upgraded into a Department of Biochemistry and Dr. Smt. Alamela Venkataraman, who was Senior Biochemist so far, has been appointed as Assistant Director in charge of the Department of Biochemistry. A Public Relations Officer was appointed.

The load of routine activities of testing bacteriological specimens and drugs continues to be heavy. It has not been possible to expand the laboratories of the Institute while the clinical and drug control activities elsewhere have considerably expanded, thus putting great strain on the available resources of the Institute.

During the year, blood transfusion campaign was inaugurated by the Chief Minister Shri Y. B. Chavan and the Institute has launched an intensive drive for blood collection, wherein, it is gratifying to note, a large number of employees have personally responded to the appeal.

The Annual Sports Day of the Sports Club of the Institute was celebrated on 22nd March 1960 when Shri S. L. Silam, Speaker of the Maharashtra Legislative Assembly presided.

A Meeting of the Indian Standards Institution, Glassware Sectional Committee, was held in the Institute in December 1960.

The Haffkine Institute Advisory Committee held its last meeting in the month of March 1960. It is necessary to record the valuable services rendered by the said Committee in tendering advice on the affairs of research and development at the Institute.

Government of Maharashtra has now constituted a State Advisory Board for Haffkine Institute to advise Government on broad outlines of research and development at the Institute under the Chairmanship of Dr. V. R. Khanolkar, Vice-Chancellor of University of Bombay. The members are : Dr. B. Mukerji, Dr. C. G. Pandit, Dr. B. B. Dikshit, Lt.-Col. S. L. Kalra, Dr. J. C. Ray, Dr. S. Govindarajan, and the Director of the Haffkine Institute as *ex-office* member-secretary.

It is sad to record the death of Shri T. P. Pednekar, Shri S. V. Kadam, Shri M. B. Khudkar and Syce Ramsunder Nasib. Members of the staff offered their continued wholehearted co-operation. The several committees looking after the various activities of the Institute functioned very effectively and enthusiastically.

But for the valuable co-operation received from all quarters, the progress reported in the following pages would have been difficult to achieve. Thanks are also due to several collaborating organisations such as Indian Council of Medical Research, Indian Council of Agricultural Research, Council of Scientific and Industrial Research and many other laboratories for their generous assistance at all times.

2. DEPARTMENT OF BACTERIOLOGY

GENERAL

The department of vaccines was renamed as Department of Bacteriology from June 1960. Dr. P. K. Jain, Deputy Superintendent, Vaccine Institute, Ranchi, received training in the preparation and testing of plague, cholera, and T. A. B. Vaccines. Dr. J. P. Sarin of the Public Health Services, U. P. worked in the department for a week to study the procedure of work followed in the department. Four trainees under the short term training scheme were also trained in the preparation and testing of vaccines.

RESEARCH

Liquid medium for T. A. B. vaccine.—Permutations and combinations of different growth promoting factors such as glycerol, sodium glycerophosphate, yeast dialysate and dl-tryptophane with casein hydrolysate as the basal medium were studied. A medium giving satisfactory growth with the strains used here

in the preparation of T. A. B. vaccine was worked out. The vaccine prepared has been found to be free from abnormal toxicity and is being tested for antigenicity by the active mouse protection test.

Cholera vaccine by submerged culture method.—Preliminary studies have been carried out to study the effect of speed of shaking and the addition of different growth promoting factors on the growth of cholera organisms in casein hydrolysate. Increase in speed of shaking from 70 to 120 rotations per minute on the rotary shaker caused no increase in growth. The addition of glucose in concentration of 0.05 per cent. to casein hydrolysate (270 mg. N/100 ml.) gives the optimum growth in 48 hours under shaking, while glycerol and sodium-B glycerophosphate in concentrations of 0.1 to 2 per cent. and 0.1 to 0.5 per cent. respectively did not cause increase in growth.

ROUTINE

During the period under review, 358,820 ml. of plague vaccine, 10,983,967 ml of cholera vaccine and 423,013 ml. of T. A. B. vaccine were issued, while, 9,297,000 ml. of cholera and 507,308 ml. of T. A. B. vaccines were produced. The total quantity of vaccines issued during the year is 11,765,800 ml. The demands for plague vaccine were mostly from Madras and Bengal, those for cholera vaccine were from Maharashtra, Bihar, Uttar Pradesh, Madhya Pradesh, Rajasthan, Mysore and Kashmir State, and those for T. A. B. vaccine mostly from Maharashtra State.

3. DEPARTMENT OF BIOCHEMISTRY

GENERAL

This is now a full-fledged department with an Assistant Director in charge. Dr. (Mrs.) Alamela Venkatraman took charge of the new post from 27th July 1960. A senior fellow of the C. S. I. R., a Government of India Scholar, and three students for M.Sc. degrees are working in this department. In addition, 33 candidates were given training for varying period of time.

RESEARCH

Serum iron levels in pregnancy.—The estimation of serum iron in normal and anemic pregnant women referred to by the Wadia Maternity Hospital was continued. Thirty-one cases of normal pregnancy and 37 cases of anemic pregnancy have been studied so far. The patients treated with intravenous iron preparations were studied at regular intervals. The results obtained so far indicate that serum iron levels in anemic pregnancy are initially low, but on treatment peak level values are obtained in about 2-3 weeks and finally normal levels are maintained.

A modified procedure for routine estimation of serum iron and iron binding capacity.—With a view to simplifying and standardising techniques for routine laboratory investigations, some of the available methods were studied. For the determination of serum iron level, it is convenient to add known amounts of the standard to the serum for accurate colour measurements. The one-piece reagent, recommended by Ramsay, minimises the number of manipulations in the procedure. For the determination of total iron binding capacity of the serum, both amberlite IRA 410 and “light” magnesium carbonate were tried to remove the excess of added iron. The latter was found preferable to the resin which is no doubt very elegant to use. Individual and pooled samples from healthy donors and laboratory workers have been used for collecting data for normal values for serum iron and total iron binding capacity by the modified procedure.

Studies on liver function tests.—Twenty-one cases of cirrhosis of liver were studied fully in addition to seven follow-up cases. The tests revealed abnormalities in most of the cases. Biopsy reports indicated either “cirrhosis of liver” or marked “fatty metamorphosis”.

Electrophoretic studies.—A detailed study has been undertaken to adopt paper electrophoresis for the routine estimation of serum protein fractions for clinical work. After some preliminary trials, it was found desirable to use Whatman no. 1 filter paper instead of no. 3 paper which is more commonly used. Normal buffer of molarity 0.075 and ionic strength 0.06, and a constant voltage of 85 for 16 hours has been found to give a clearer separation and better reproducible results. Albumin trailing is very much less and the main fractions agree closely well with those obtained by chemical separation using various concentrations of sodium sulphate.

Studies on some inborn errors of metabolism.—Studies on metabolic disorders which are known to retard normal mental growth in children have been undertaken. Excretion of some abnormal components such as phenylpyruvic acid, hydroxyphenylpyruvic acid, galactose, sucrose, and large amounts of certain branched chain amino acids, have been found in some cases of mentally deficient children. It is planned to test all the available children in Bombay who show signs of mental under-development.

Studies on bacterial metabolism.—Preliminary work on the standardisation of casein hydrolysates prepared in the Media section has been started in connection with the proposed investigations on bacterial metabolism. The immediate work on hand involves the quantitative estimation of the different amino acids in casein hydrolysate by the paper chromatographic techniques.

Blood group substances from meconium.—In connection with studies in blood group substances from meconium, a thorough chemical and biochemical study of the starting material has been undertaken. The Wadia Maternity Hospital is kindly supplying the necessary samples. The study would involve

elementary analysis, identification of protein fractions, sugar and sugar derivatives, enzymes if any, and isolation and purification of the active blood group substances.

Biosynthesis of proteins.—The uptake of methionine by diabetic liver was found to be slightly higher than that by the normal liver. Insulin *in vivo* did not have any effect either on the normal or diabetic uptake. The diabetic animals for the experiment were obtained by injecting the rats with alloxan (175 mg./kg.) subcutaneously.

Biosynthesis of glucose-6-phosphatase was studied by microsomes and pH 5 enzymes system. The enzyme was assayed using glucose-6-phosphate as the substrate and estimating the inorganic phosphate liberated. After proper standardisation of the method, various co-factors affecting the protein biosynthesis will be studied.

ROUTINE

During the year under review, the department continued its usual activities of examining clinical materials from hospitals and private clinics. The work load from the local Government hospitals is getting reduced as some of the hospitals have made their own arrangements for carrying out their routine biochemical estimations. The total number of analyses carried out during the year was 6,835 as against 8,709 in the previous year. However, the amount of special work done has shown an increase. For the sake of better organization the tests have been regrouped as of simple "routine" and of special category with the idea that the former will be taken up by the Government Hospital Laboratories, thus enabling the department to concentrate more on specialised types of tests. During the year 1960, 839 special tests were carried out consisting of the following :—

Name of the test—

Basal Metabolic Rate	..	..	..	..	93
Gastric Analysis	..	..	..	..	71
Glucose Tolerance test	..	..	..	..	71
Kidney Function	..	..	..	..	104
Lange's colloidal Gold Cureve	..	..	..	..	108
Serum Potassium	..	..	..	..	19
Serum Sodium	..	..	..	..	24
Urinary 17-Ketosteroids	..	..	..	..	22
Serum Iron	..	..	..	..	167
Serum Transaminases	..	..	..	..	122
Urinary, Copper, Mercury, Lead	..	..	..	..	7
Urinary Catecholamines	..	..	..	..	1
Serum Proteins Fractions-Chemical	..	..	..	..	27
Serum Proteins Fractions-paper Electrophoresis	..	..	..	..	3

DEPARTMENT OF CHEMOTHERAPY

GENERAL

The activities of the Department increased in research, production, testing and training programme. The research work carried out during the year (based on the general plans submitted for the year 1959-1960) comprised mainly of the studies in the synthesis of hypoglycemic agents, diuretics, anti-tubercular compounds, anticonvulsants and central-nervous-system stimulants. Hormone study initiated in the department in the previous year, was continued and with the arrival of Russian expert Madam Zvereva, it was possible to carry out pilot plant trials for insulin and laboratory studies on the isolation of other glandular products such as ACTH, thyroxin, adrenalin, pepsin, etc. Work on the isolation of digitoxin and other glycosides from indigenously grown *Digitalis purpurea*, studies on crystalline fractions from *Veronica anthelmintica* and tuberculostatic activity of *Capparis moonii* fruits were continued. The Drugs screening unit carried out bacteriostatic studies against *M. tuberculosis*, *P. pestis*, *V. cholerae*, and *S. typhosa*. Two patent applications for the preparation of oral antidiabetics were filed in India. The production and sale of sulphamonomethoxime and vitamin tablets, pemon, etc. showed more than twenty-five per cent. increase over the previous year. Fifteen science graduates took training in the different sections of the department. Eight post-graduate students continued work on their research problems for Ph.D. and M.Sc. degrees. Two Unichem fellows, one Government of India scholar, one Government of Maharashtra scholar and two C. S. I. R. research fellows continued to work in the department.

RESEARCH ACTIVITIES

Compounds with possible oral hypoglycemic activity

(a) *Synthesis of monosulphonylureas*.—The work on sulphonylureas started last year was continued and 45 new benzene sulphonylalkylureas were prepared by the process developed in the department and patented. These compounds were tested in the department of Pharmacology and out of these, nine compounds have been found to possess potent antidiabetic activity in the animal experiments. The compound R-94, which showed prolonged activity, was clinically evaluated in the Diabetic Clinic of K. E. M. Hospital, Bombay by Dr. J. C. Patel on 18 diabetic patients. He found this compound to possess more potent hypoglycemic effect than tolbutamide. However, the treatment of 8 patients had to be discontinued because of side reactions consisting of tinnitus and deafness.

(b) *Synthesis of disulphonylureas*.—Twenty, 1, 3-bis (N-alkyl or aryl ureido-N¹-sulphonyl) benzene or substituted benzene derivatives have been synthesised and are being screened for their hypoglycemic activity in the Department of Pharmacology. So far, two compounds have been found to possess good hypoglycemic activity.

Compounds with possible oral diuretic activity

(a) *s-Triazine derivatives*.—The work on the synthesis of *s*-triazine derivatives was continued. Out of the eighteen compounds synthesised, 2-(*m*-chloroaniline)-4-amino-*s*-triazine (R-37) was found to possess potent diuretic and saluretic activity in rats and dogs by the Department of Pharmacology of Seth G. S. Medical College. This compound was therefore prepared in large quantity, converted into tablets and submitted for clinical evaluation to Dr. D. D. Vora at the Lokamanya Tilak Municipal General Hospital, Sion.

(b) *Triazoles and Thiadiazoles*.—Forty 1-alkyl-2-aryl or pyridyl-1, 3, 4-triazole-5-thiones and fifteen 2-aryl or pyridyl 5-alkyl amino-1, 3, 4-thiadiazole derivatives were prepared from appropriate thiosemicarbazides. These triazole derivatives are being tested for diuretic activity in experimental animals in the Department of Pharmacology, Seth G. S. Medical College.

Compounds with possible antitubercular activity

(a) *Thiazolyl and pyrimidylthioureas*.—In continuation of the work reported previously, twenty eight thiazolyl and thirty two pyrimidyl thiourea derivatives have been synthesised and screened for antitubercular activity *in vitro* against H-37 Strain of *M. tuberculosis*. Eight pyrimidyl derivatives show inhibitory activity in concentrations of 2 ug/ml., and 12 of the thiazolyl group in concentrations of 20 ug/ml. Further work on study of the most promising compounds is in progress.

(b) *Semicarbazide and hydrazide derivatives*.—A few semicarbazide and hydrazide derivatives of 2-methyl-1 : 4-napthoquinone (Vit-K3) were prepared as potent anti-tubercular agents. Out of 12 such derivatives, two have shown *in vitro* tuberculostatic activity at a concentration of 2 ug/ml.

Central nervous system (CNS), stimulants

(a) Recently several hydrazine and hydrazide derivatives such as isonicotinoyl-2-isopropylhydrazine (iproniazide), 1-phenyl-2-hydrazino-propane (Catron) and 1-phenyl-2-hydrazine ethane (phenelzine) described as inhibitors of monoamine oxidase (M.A.O.) are reported to be much more potent as C. N. S. stimulants, used as 'psychic energisers' and have been found to give considerable relief in angina pectoris also. 2, 4-Diamino-5-phenyl thiazole (daptazole) a potent analeptic and respiratory stimulant drug, is being used clinically in cases of morphine poisoning. In anticipation that introduction of a hydrazine moiety in the thiazole ring may lead to compounds having potent C. N. S. and respiratory stimulant properties, a series of 2-acylhydrazino-4-amino-5-phenyl thiazoles have been synthesised, the acyl groups being formyl, acetyl, propionyl, butyryl, benzoyl, *o*-hydroxybenzoyl, *p*-hydroxybenzoyl, *m*-chlorobenzoyl, *p*-chlorobenzoyl, *m*-nitrobenzoyl, *p*-nitrobenzoyl, *o*-methoxybenzoyl, *p*-methoxybenzoyl and *m*-methylbenzoyl. All these

thiazoles have been obtained as benzene sulphonate salts and attempts to convert them into hydrochlorides lead to 2-hydrazin-4-hydroxy thiazoles. All these compounds are being tested.

(b) Gamma-aminobutyric acid (G. A. B. A.) produced in the brain from glutamic acid has a considerable inhibitory action on the central nervous system and thiosemicarbazide ($\text{NH}_2\text{-CS-NH-NH}_2$) reduces the level of G. A. B. A. in the brain and thereby stimulates C. N. S. activity and even produces convulsions. A large number of acyl, alkyl and aryl substituted thiosemicarbazide derivatives have been synthesised as potent non-convulsant C. N. S. stimulants.

(c) A series of 2-alkylamino-4-amino-5-phenyl-thiazoles and 2-arylamino-4-amino-5-phenylthiazoles have been synthesised to study the correlation of structure and biological properties in this type of compounds. All these derivatives are being tested for their respiratory stimulating properties and as morphine antagonists.

Synthesis of 2-pyrazolin-5-one derivatives as possible analgesic :—The work on the synthesis of 2-pyrazolin-5-one derivatives was continued and a series of 3-aryl-4- (B-hydroxyethyl)-2- pyrazolin-5-ones has been synthesised, the aryl group being either phenyl, p-chlorophenyl, p-methoxyphenyl, 3, 4, 5-trimethoxyphenyl, p-nitrophenyl, or m-nitrophenyl.

Studies on Indigenous Drugs. Digitalis purpurea : A suitable method for the extraction of active glycosides from indigenously grown *D. purpurea* has been worked out which gives good yields of cardiotonic glycosides and has the advantage of using cheaper solvents and less manipulative operations than the method described in literature.

V. anthelmintica.—In view of the activity *in vitro* shown against *V. cholera* by a crystalline product isolated from *V. anthelmintica* previously in this department, isolation of sufficient material was undertaken for its chemical characterisation. During this work, one more crystalline substance (m. p. $74\text{--}75^\circ\text{C}$) containing only carbon, hydrogen and oxygen has been isolated. This substance is labile in nature, a characteristic which was noted in the previous compound also. Infra red spectra of this compound also indicated the presence of a lactone ring and close similarity in the structure with the previous one. This is being further investigated.

Estimation of manganese in clinical material.—On behalf of Manganese Poisoning Inquiry Committee of Government of India, the department undertook the estimation of manganese in clinical materials such as serum, blood, C. S. F., urine, stools, etc. After a trial of various methods, a modification of the method of Weise and Johnsons of using benzidine under acid condition was found to be satisfactory, and with this method, a large number of samples from normal and manganese poisoned patients, received from Dr. N. H. Wadia of Sir J. J. Group of Hospitals, have been assayed.

(b) *Urinary output of 5-Hydroxyindoleacetic acid in normal pregnancies and complications of pregnancy.*—Serotonin (5-hydroxytryptamine) is known to produce increase in blood pressure, contractions of smooth muscles and oedema. Since these symptoms are present in the complications of pregnancy, 24 hour 5-hydroxy-indoleacetic acid urinary output in normal pregnancies and complication of pregnancy were measured. The work is being continued in collaboration with Dr. M. N. Parikh, M.D., D.A. of Wadia Maternity Hospital.

Hormones and glandular products. (a) In connection with the proposal for the establishment of a hormones and glandular products unit in the public sector, data regarding the average weight of different glands and the contents of different hormones in these were needed. A large number of glands were collected and the weights were recorded with the kind help of Bombay slaughterhouse authorities. With the arrival of Madam Zverava, from All Union Institute of Experimental Endocrinology, Moscow, on behalf of the Ministry of Commerce and Industry, Government of India, it was possible to study the various processes for the isolation of crystalline insulin, ACTH, adrenaline, thyroxine, pepsin etc. under her able guidance during her stay here from 7th March to 12th May, 1960. The extraction of insulin from different proportions of a mixture of sheep and cattle pancreas was studied and the optimum ratio for obtaining best yields of crystalline insulin was arrived at in the laboratory and pilot-plant scale. The yields of ACTH, adrenaline, thyroxine and pepsin obtained from glands collected from Bombay slaughter house, were found to be comparable to those available in other countries.

(b) *Assay of insulin preparations.*—In view of the elaborate and time consuming biological assay of insulin, work was undertaken to standardise insulin assay by a physico-chemical method. The insulin from the commercial samples of injectibles was separated by circular paper chromatography and assayed by developing the colour and measuring its intensity spectrophotometrically. It has been shown that the results of this physico-chemical method are quite comparable with biological assays (mouse-convulsion method). To ascertain whether the presence of the degradation product of insulin present in time-barred or improperly stored samples would interfere in the paper chromatographic method of assay, such samples were also studied. Both the methods gave identical results thereby indicating that the degradation products do not interfere in this physico-chemical method of insulin assay.

Biological testing (drug screening). (a) *In vitro testing of compounds.*—Out of 111 compounds tested against H 37 Rv strain of *M. tuberculosis* in Youman's medium with horse serum, only 31 compounds showed activity in concentration below 20 ug./ml. in the preliminary screening. These were retested in still higher dilutions and seventeen compounds showed activity in concentration below 10 ug/ml. About 40 compounds have been tested for activity against *V. cholerae*, but none has shown activity upto a concentration of 200 ug/ml. None of the 20 compounds tested for activity against *S. typhosa* showed any activity

upto a concentration of 20 ug/ml. *Capparis moonii* juice kindly sent by M/s. Vats and Co. was tested against *M. Tuberculosis* (H37Rv strain), *V. cholera*, and *P. pestis*. Anti-tubercular activity was noted in dilutions upto 1:50 in Youman's medium with horse serum and modified Yamane's liquid medium. Seitz filtered and heat-sterilised samples showed the same activity. Activity against *V. cholerae* was noted at 1 : 10 dilution, but did not show any activity against *P. pestis*. However, the samples of juice as received were already stored for several months at room temperature and had fermented. Fresh sample is awaited for further experiments.

(b) *In vivo testing of compounds—Toxicity experiments.*—The compounds R-153, R-154 and R-349 which had shown good tuberculostatic activity were broadly screened for their chronic toxicities, using the institute inbred mice. The first two compounds were tolerated in the concentration of 50 mg/kg.

(c) *Tuberculostatic activity of Capparis moonii fruits.*—Two varieties of these fruits were used for feeding guinea pigs and their serum was tested for tuberculostatic activity by addition to Youman's medium inoculated with H37Rv strain of *M. tuberculosis*. Only the Konkan variety showed tuberculostatic activity.

PRODUCTION

During the year under review, the department continued to manufacture and supply Vitamin, Sulpha drugs and Quinine preparations to the hospitals and dispensaries of Government and Municipalities, and to Employees' State Insurance Scheme. All demands were fully met. The table shows the products supplied during the year.

Serial No.	Name of the Products.	Number of tablets supplied.
1	Vitamin AD tablets	9,608,500
2	Vitamin AD (Spl.) tablets	77,000
3	Vitamin A Forte tablets	145,000
4	Vitamin AD Forte tablets	1,101,450
5	Vitamin BC tablets	15,818,934
6	Vitamin BC No. 3 tablets	755,400
7	Vitamin B1 tablets	1,298,824

Serial No.	Name of the Products.				Number of tablets supplied.
8	Vitamin B2 tablets	..	..	..	173,408
9	Vitamin C tablets	..	..	..	2,164,804
10	Acid Nicotinic tablets	...	..	..	479,174
11	Nicotinamidet ablets	...	..	..	49,850
12	Multivitamin tablets	..	..	..	15,541,218
13	Vitamin K tablets	..	..	..	64,250
14	Magnesium Trisilicate tablets	..	..	..	346,200
15	Sulphathiazole tablets	...	..	..	383,800
16	Sulphadiazine tablets	...	..	..	7,926,250
17	Sulphaguanidine tablets	..	..	..	4,315,725
18	Quinine Sulphate tablets	..	..	..	511,225
Total					64,161,012
19	Sulphanilamide paste	..	..	..	597 lbs.
20	Pemon	..	..	..	20, 826½ lbs.
21	Sulphathiazole powder B. P.	..	..	..	89, 168 kg.
22	Solapsone Strong B. P. C. Solution	..	..	..	586 × 50 ml.

During the period under review 809·8 kg. of sulphathiazole powder were manufactured and also intermediates equivalent to 135 kg. of sulphathiazole. In addition, 83·07 kg. of B. P. grade quinine sulphate was obtained from substandard quinine sulphate, after purification.

ANALYTICAL SECTION

The analytical section of the department analysed 916 samples of raw materials and finished products during the year ; 927 microanalysis of compounds synthesised in the department were carried out.

5. DEPARTMENT OF CLINICAL PATHOLOGY AND DIAGNOSTIC REAGENTS

GENERAL

Four members of the Department have registered for post-graduate degrees, one for Ph.D and others for M.Sc. degrees. As in the previous years, the students of the Technicians training course were given training in Clinical Pathology techniques. In addition, 13 candidates were also admitted for Short term training of three months duration.

RESEARCH

Prevalent serological types of urinary and other E. coli in Bombay.—Bacteriological and serological study of 67 typical *E. coli* strains isolated from 152 urine samples, 14 vaginal swabs and 14 rectal swab cultures were made. Of the 41 urinary strains 25 (61 per cent.) were agglutinable. The most common serotypes were 086, 0112 and 0126 comprising more than 50 per cent. of the total.

Infantile and childhood cirrhosis.—Various liver function tests done in 28 cases revealed abnormalities in most of the pediatric hepatic diseases. The cases in which liver biopsy reports were available showed changes of either "Cirrhosis of the liver" or "marked fatty metamorphosis".

Bacteriophage types of Salmonella typhi and Salmonella paratyphi A as encountered in Bombay City.—Results of 271 typed strains reveal that phage A is predominant, frequency of D1 is increasing, and hitherto unnoticed phage types like D6, J1, H1, T & 28 are now encountered. The position of other types is unchanged. Five new phages were isolated by lysogenic studies.

They belonged to group '0' phages. Degraded cultures are increasingly encountered.

"Mycological flora of otomycosis in Bombay".—A mycological cultural study of 32 otomycosis cases and 25 mycologic cultures of external ears of normal persons has been reported. Findings are in agreement with these of others that the common species responsible for infection are *Aspergillus*, *Mucor* and *Rhizopus*.

Studies on the Antigenic structure of typhoid-paratyphoid group under partial anaerobiosis.—Attempts to prepare a concentrated antigen for bed-side slide-agglutination test have not been so far successful. Further studies are in progress.

Studies of the alimentary canal of the different rat-fleas.—Further studies about the feeding and transmission potential of normal and infected fleas are being carried out in collaboration with the Entomology Department.

Serological typing of E. coli.—In collaboration with (1) Polio Research Unit, G. M. College, Bombay, (2) Gastro Enterology unit T. N. M. College, Bombay and (3) Gynaecology Research Unit ; K. E. M. Hospital, Parel, Bombay. 220 *E. coli* strains have been typed.

Bacterial flora of the cervix in sterility cases of Indian women.—In collaboration with the Superintendent, Cama and Albless Hospital, Bombay, 66 cases have been investigated.

Biochemical and histological studies of infantile cirrhosis cases.—In collaboration with Dr. S. M. Merchant, Bai Jerbai Wadia Hospital for Children, Parel, Bombay, 28 cases were studied in detail during the year.

ROUTINE

Arrangements are progressively being made for carrying out specialised types of diagnostic reference work. The department now carries out typing of *Salmonella* cultures.

Nichol's virulent strain of *T. pallidum* is maintained and the Immuno-Fluorescence Antibody (IFA) technique is being standardised for diagnosis of syphilis. Nineteen passages were carried out intratesticularly in rabbits and sufficient antigenic material has been collected to start IFA test as early as possible.

The venereal diseases Reference Laboratory section is proving useful. Guidance was given to a number of workers, students and laboratory personnel in regard to the various standard techniques and reagents.

Standardisation and testing of referred and selected samples of sera for streptolysin 'O' antibody level are being carried out. One hundred sera were tested during the year.

Mobile Unit.—The technicians of this unit have been trained. Equipment is also partly collected. It is expected that with the recruiting of the Medical Officer, this unit will be put into field work soon.

General Bacteriological work.—Samples of articles of food and beverages received from the Forensic Science Laboratory, Bombay, are being examined for evidence of toxin or poison. Eleven such exhibits of medicolegal cases were bacteriologically tested during the year. In February 1960, symptoms of food poisoning occurred in a large number of workers using a staff canteen in the suburb. A very detailed investigation was carried out in this connection on behalf of the organisation. The department continued the general activities of (i) conducting bacteriological, serological and biological tests on all clinical materials received from Government hospitals and other Institutions; (ii) preparation and supply of various diagnostic reagents, stains and snake-venom solution; (iii) carrying out of histopathological examinations on biopsy materials and pathological tissues; (iv) preparation of autogenous vaccines, and (v) conducting special tests like pregnancy tests, Rideal-Walker test, Frei's and Tuberculin tests etc. All essential tests in connection with infectious diseases like diphtheria, cerebrospinal fever, enteric group of fevers, typhus, plague, cholera and tuberculosis, were carried out free of cost.

Histopathological section.—During the year under review, 3803 pathological specimens received from different hospitals in the State of Maharashtra and a few from outside the state were examined and reported upon. These included brain tissues from suspected rabid animals, neoplasma, gynaecological conditions and some rare and interesting pathological specimens. The total number of blocks cut were 5250.

Some of the interesting specimens received during the year were as follows :—

Adamantinoma ; Malignant type of teratoma of testis ; Renal cell carcinoma ; Fibroid of the ovary ; Chorion Carcinoma of uterus ; Pleomorphic adenoma of the lacrimal gland ; Osteoid Osteoma ; Plasma cell mastitis ; Dysgerminoma of the ovary ; Retinoblastoma ; Granulosa cell carcinoma of the ovary ; Hurthle cell adenoma of thyroid ; Tuberculous thyroiditis ; Follicular adenocarcinoma of thyroid ; Plasma cell myeloma of the mandible ; Sweat gland carcinoma ; Neuroblastoma ; Fibromyoma undergoing sarcomatous changes ; Malignant teratoma of the ovary ; Fibrosarcoma of breast ; Schwannoma of ulnar and nerve ; Ewing's sarcoma of shoulder and Hydatid cyst of the kidney.

Typhoid scheme.—Calls received from the various private medical practitioners in the city of Bombay were attended to. All the blood samples collected were as usual put up for clot culture and triple Widal tests. There were 437 calls for blood collection. Of the 2010 Widal and clot culture tests carried out, Widal test was positive in 474 cases, 156 cultures were positive of which 135 were of *S. typhi* and the remaining 21 were of *S. paratyphi A*.

Routine Examinations.—During the period under review, the following tests were carried out :—

1. SEROLOGICAL EXAMINATION

(i) Wasserman Test	..	..	..	..	429
(ii) Kahn Test and V. D. R. L. test	..	..	..	..	478
(iii) Triple Widal test	..	..	..	..	2010
(iv) Agglutination test for—					
(a) Typhus	..	..	..	..	1660
(b) Brucellosis	..	..	..	..	1660
(c) Leptospirosis	..	..	..	..	32
(d) Paul-Bunnell Test	..	..	..	..	4

II. BACTERIOLOGICAL AND BIOLOGICAL TESTS

(i) Clot culture for enteric group	..	..	..	1660
(ii) Urine culture for pathogenic organisms	..	..	..	60
(iii) Throat-swab for Diphtheria	..	..	..	274
(iv) Stools and rectal swabs for culture	..	..	..	18
(v) Sputum and body fluids for AFB for smear, culture and animal inoculation test	..	..	..	670
(vi) Blood culture routine	..	..	..	35
(vii) Preparation of autogenous vaccine	..	..	..	55
(viii) Bone Marrow	..	..	..	6
(ix) Material for smear and examination (Presence of pathogenic organisms like G. C. etc.	..	..	..	25
(x) Stools	..	..	..	540
(xi) Urine	..	..	..	446
(xii) Prothrombin Time Estimation	..	..	..	
(xiii) Pregnancy test	..	..	..	1143
(xiv) Rideal Walker test	..	..	..	90
(xv) Antibiotic Sensitivity test 1(p)	..	..	..	78(u)

Diagnostic Reagents.—This section is proving useful. The demands for Group sera and Kahn antigen were increasing. Casoni's antigen (Hydatid fluid) has been added as a fresh supply item, in view of several demands for this reagent.

The production and issue during the period under report are :—

1. Killed suspensions for Widal and Weil Felix test	..	..	..	265,490 ml.
2. Blood grouping sera	..	..	..	18,464 „
3. High Titre sera and Amboceptor	..	..	..	1,692 „
4. Kahn Antigen	..	..	..	12,240 „
5. W. R. Antigen	..	..	..	740 „
6. Russell's Viper and cobra Venom solutions	..	..	..	1,280 „
7. Biological stains	..	..	..	502,800 „

6. DEPARTMENT OF ENTOMOLOGY

GENERAL

Capt. D. C. Cavanaugh from the Infectious Diseases Section, Walter Reed Army Institute of Research, Washington, D. C. worked in the department from 24th April 1960 to 20th September 1960. He collaborated in the work on some epidemiological aspects of plague in Bombay. The Assistant Director was deputed to NEFA under a defence ministry assignment for a period of three weeks. He attended the "Entomological and Animal Pests of Plant Committee" of the Indian Council of Agricultural Research, New Delhi. He participated in the symposium on the use of "Radio-isotopes in Entomology" sponsored by the International atomic energy agency in Bombay and the symposium on the "Termites in the Humid Tropics" organised jointly by the Zoological survey of India and the UNESCO. The Junior Scientific Officer toured East Khandesh District to collect the information about snakes for the Bombay District Gazetteers. The account of snakes for the East Khandesh was completed. A new wing of the laboratory for the venomous animal unit was declared open in the month of January 1960. This laboratory would initially study snake behaviour and make arrangements for the extraction of venoms. Three students continued their work for Ph.D. and one for a M.Sc. degree. Three new students registered for their Ph.D. degrees. Lectures on reptiles were delivered to M.Sc. students in zoology at the Bombay University.

RESEARCH.

Studies on hormones in Musca nebulo.—Histological studies on some glands of flies during the different pupal stages showed that the corpus allatum remains inactive during the pupal period. The corpus cardiacum on the other hand shows great secretory activity, particularly at the time of emergence. Large stainable colloidal particles are observed in the cells of the corpus cardiacum and in the oldest pupae these colloidal particles are observed to have been accumulated in the intercellular spaces, which are closely associated with the aortic wall. This presumably represents their mode of entry into the haemolymph. The peritracheal glands degenerate completely by the time the adult is ready to emerge or within a few hours after emergence. From the histological evidence therefore it may be concluded that the corpus cardiacum plays an important role in the differentiation and growth of the imaginal buds, and also in the mechanism of emergence. Studies on the neurosecretory cells of the adult female housefly confirm the histological findings that the neurosecretory material increases during ovary development. The acid Alcian Blue method of Admas and Sloper showed that in young females the neurosecretory cells contain little secretory material; but in the older females the intensity of staining of this material increases considerably and then falls by the time oviposition has begun. This cyclical change is probably associated with ovary

development. Further studies on the R. N. A. and D. N. A. contents and also on the cystine content of the N. S. cells and the corpus cardiacum and c. allatum complex are being done. Confirmatory experiments have also been done to establish the fact that extirpation of corpus allatum from young females prevents maturation of the ova, and transplantation of the corpus allatum from a mature female will induce ovary development, in such extirpated females. The ring gland from early larvae can also stimulate ovary development in the extirpated female, but ring glands from the older larvae failed to promote ovary development. On the other hand, the second series of experiments on the extirpation of the N. S. cells, failed to confirm the previous results. Sections of the egg during different developmental stages have been taken to study the origin and development of these endocrine glands.

Studies on Bombay Rats.—Collection of different kinds of rats from Kamatipura, Mahim and Dharavi, have shown that the percentage of *Bandicote bengalensis* was on the increase. To check whether this is a true picture of the state of affairs, studies on the frequency of rat population and the rat migration if any were started in four areas. These experiments were done in the Haffkine Institute compound in Parel, Khetwadi, housing area and field area in Bhandup. Initially trial was given to wonder traps but later only the new traps designed in this department were used. This new trap collects only one rat and big bandicots find easy entrance into the same. So far 470 rats have been trapped during these experiments. They have been marked, classified, sex ratio determined, and ectoparasites recovered. The point of interest in the first two experiments is that in the Parel housing area shrews predominated and there were four cases of migration noted in house rats in the Khetwadi area. The point of interest in the last two experiments is that in both the cases the rat *Rattus rattus* came first in the traps. When *B. bengalensis* started coming, the number of *Rattus rattus* decreased. Initially came the male rats followed after some days by young ones and lastly came female rats. Wonder traps do not catch any *B. gigantea*. Only the younger *B. bengalensis* are caught in it. Nests of *B. Bengalensis* and *B. indica* have been dug out and the young ones recovered. These experiments will continue for quite a time. Having studied the rat burrow pattern in the laboratory, studies are in vogue in Mahim area to study this in nature. So far 176 burrows have been excavated, of which 174 are indicated to be those of *B. bengalensis* and 2 of *B. indica*. In these experiments only one *B. indica* and five *B. bengalensis* have been recovered alive from inside the burrows. Of these rats, only one was adult male *B. bengalensis*, and the rest were females. There were 141 adult fleas and 219 flea larvae recovered from these burrows. In the adult fleas 77 were males and 64 females. All the fleas were *X. astia*. A large number of mites have been recovered from the burrows and these are being identified. The resistance of rat fleas collected from the dock area and Kamatipura area to varying doses of DDT and BHC is being tested by the petri-dish technique on a comparative basis with W. H. O. method of impregnated papers. Studies

have been started on the sucking potential, blocking potential and the transmission potential of the males and females of *X. cheopis* and *X. astia* at a standard temperatures of 26° C, after twenty-four hours starvation and a blood meal for definite number of minutes.

Plague epidemiology.—Plague epidemiological studies were done in collaboration with Capt. D. C. Cavanaugh of Walter Reed Army Institute of Research, Washington. About 900 rats belonging to species *Rattus rattus* and *B. bengalensis* were bled during the period from April to September, 1960. The sera of these rats were tested for antibody to *P. pestis* and *P. pseudotuberculosis* antigens by the agar-gel double diffusion Oudin reaction. It was noticed that the sera from the more resistant rats in Kamatipura displayed antibody to *P. pestis* and *P. pseudotuberculosis* somatic antigens which are common to both species but not to the specific capsular antigen of *P. pestis*. *B. bengalensis* and *Rattus rattus* from these areas were subjected to a standard challenge dose of *P. pestis*. The results indicate the differences in the percentage mortality of both the species in the two geographically separated areas. They were then submitted to a standard challenge dose of 10^{-2} , 10^{-3} , 10^{-5} , 10^{-6} , dilution of *P. pestis* (195 p. 5 in 0.2ml). The results indicate that as compared to a laboratory mice, *B. bengalensis* is 45 per cent. and *Rattus rattus* 96 per cent. resistant to plague. The resistance of *Rattus rattus* and susceptibility of *B. bengalensis* in Bombay and a number of towns in India to *P. pestis* infection is well known. It is of interest to see that while *B. bengalensis* is replacing *Rattus rattus* in Bombay; it is also in the process of acquiring resistance. The experiments to see rat mortality in *Rattus rattus* and *B. bengalensis* from Kamatipura and Mahim to a standard dose of *P. pestis* in each month are continuing to see whether there are any significant differences in the percentage mortality from these two areas for different months.

Venomous animals.—Venomous animal food experiments, done with an artificial food, have shown that the young ones of Russell's viper could be reared upto an age of 2 months when they could take to a solid food of frogs. The snake behaviour experiments in the snake farm and experimental cages have shown that Cobra always remains hiding during the day and with the least noise decamps into the hide-out. The Russell's viper sits out in a shade well within sight, but under a definite shady camouflage. They are not disturbed by noise unless someone is pretty close when they start hissing. In the case of kraits they remain hiding in crevices but many a time a part of their body is exposed to light to which there is no reaction. They are disturbed by both light and noise from close quarters. Two species of scorpions, *Palamnaeus gravimanus* and *Buthus tamulus* have been reared up on cockroaches. There was a heavy mortality with mutton pieces and glucose saline. This was reduced by a diet of cockroaches and sugarcane bagasse. *Buthus tamulus* has been

bred in number in the laboratory. A modified technique to extract the venom of scorpions by electricity has been evolved to give a continuous supply of scorpion venom for experiments.

ROUTINE

During this year the department dealt with 9,503 visitors that came to see the venom extraction demonstration. Of these 8,015 are from Institutions like technical colleges and schools. The number of enquiries for the identification of snakes were 45. There were 77 enquiries for the supply of snake information. The total number of snakes purchased was 15,236 from Ratnagiri, Kolaba, North Satara, Ahmednagar and Yeotmal districts of the State of Maharashtra.

The following is the venom output of snake venom for the year 1960 :—

Types of snakes.					Venom produced in gm.	Venom supplied in gm.
Cobra	..	..	..	..	21·8308	65·2362
Krait	..	..	..	..	11·6429	10·3048
Russell's viper	..	..	..	..	45·4258	9·6090
Echis carinatus	..	..	..	..	39·0421	28·2801
Total					117·9416	113·4301

The total amount of venom produced from the scorpions was 4·0855 gms. ; the average output per scorpion was 0·8 mg. and 0·47 mg. for the two varieties, *Palamnaeus* and *Buthus* species respectively. The department handled 20 enquiries about rat and insect control. The sanitation of the Institute was handled by this department, including the control of rats and cockroaches within the premises.

Bombay Municipal Rat Section.—Experiments on the susceptibility of Bombay City rats to plague infection by injecting a standard test infective dose showed results similar to last year.

During the year 39 dead rats, 56,901 live rats from the City and 4,593 live rats from the Bombay Port Trust area were examined for plague. No plague infection was detected.

7. DEPARTMENT OF IMMUNOLOGY

GENERAL

The department has been feeling the need for expansion of production facilities as the demands for antisera and toxoids far exceeded its production capacity. Antisera production was adversely affected to some extent during the year by the threatened African horse sickness against which our horses had to be vaccinated thus suspending the immunization schedule for sometime. There are plans to develop the Pimpri Farm near Poona to meet the expanding needs of the department. Forty-four acres of land adjacent to the farm have been acquired during this year. A bleeding chamber for immunization and bleeding of horses, and additional stables are expected to be ready in the coming year. Dr. K. V. Kulkarni, who was on deputation for higher training to U. K. under the Colombo Plan returned and took charge on 1st October 1960.

RESEARCH

Purification of Antisera by "phenol" method.—The method of purification of horse antitoxic sera by fractional precipitation with ammonium sulphate at 1.5 per cent. phenol concentration was further improved. One fraction precipitating between 15 and 20 per cent. saturation with ammonium sulphate had slightly higher purity than that obtained by the Gibson-Murdick procedure in routine use at this Institute; the second fraction precipitating between 20 to 35 per cent. saturation had nearly twice the activity. The total yields were also better than the routine method. The purified fractions were analysed for homogeneity by gel diffusion and immunoelectrophoresis using rabbit anti-horse sera. The antisera produced by the routine method was found to contain 13 antigenic components, the 15 to 20 per cent. fraction had 9 and the 20 to 35 per cent. fraction only four. Comparative stability studies of diphtheria antisera purified by different methods were carried out over a period one year. Results show that the phenol purified antisera was more stable than the antisera produced by the routine method.

Distribution of antitoxic activity in the different electrophoretic fractions of antitoxic horse sera.—A pooled sample of antidiphtheria horse sera and also samples of horse antidiphtheria pseudoglobulin fraction from different batches were subjected to agar electrophoresis according to Grabar's method. It was found that nearly 84 per cent. of antitoxic activity was in the beta globulin fraction and the remaining activity was distributed equally between the alpha and gamma globulins. The above results were further confirmed by immunoelectrophoresis of the antisera using rabbit antihorse sera on one side and diphtheria toxin on the other side. In the case of the pepsin digested horse antisera however, 90 per cent. of the antitoxic activity was found in the gamma globulin and 10 per cent. in the beta globulin. Immuno-electrophoresis of the samples showed that beta-globulins of horse sera were converted into proteins having the mobility of gamma globulins after digestion with pepsin.

Study of degradation of horse serum proteins with pepsin.—Horse serum albumin was prepared in electrophoretically pure form by the method of Kekwik, and of Korner and Debre. Pepsin digestion of the albumin was studied at short range of pH between 1 and 4.5 using purified pepsin and crystalline pepsin. Samples were taken out during degradation and analysed by immuno-electrophoresis. Results show that pepsin degrades albumin into 3 components during the early stage of degradation, which react with rabbit anti horse albumin. Later on, they are further degraded into smaller molecules which no longer show precipitation with antisera to whole albumin. These results will form the basis for the purification of antisera by pepsin digestion.

Study of protein-protein interaction in sera.—When normal human sera or normal horse sera is heated at temperatures from 55° to 70° C, it was found that albumin combines with the other proteins of sera especially with the alpha and beta globulins and also with gamma globulins to some extent. These interactions could be inhibited by treatment of sera with iodoacetate and accelerated by reduced glutathione. Results suggest that the free SH groups of albumin and other serum proteins react bringing about protein-protein interaction by sulphhydryl-disulphide exchange. The effect of various reagents on protein-protein combination and on the antitoxic activity of diphtheria horse sera and the diphtheria antitoxic gamma globulin have been determined. The results are likely to throw light on the deterioration of antitoxic sera during storage.

Antigenic analysis of Cholera Vaccine.—Cholera vaccine contains the protective antigens in the cell-free supernatant, as tested by the mouse protection method. The supernatant of Inaba vaccine when analysed for antigenic composition was found to contain 4 heat stable 'O' antigens and 7 heat labile 'H' antigens. Cholera vaccine heated for 2 hours at 100° C was found to have the same protective power as the unheated vaccine. The protective antigens therefore belonged to the 'O' group. Different methods of extraction and purification of the antigens were tried and the protective power of these extracts determined. The ethylene glycol extract of heated vaccine had the highest protective power of 5 micrograms compared to other extracts and contained only two antigens of 'O' group as shown by gel diffusion test.

Antigenic analysis of Clostridia.—The clostridia group of organisms, viz tetanus, welchii, oedematiens and septique, produce the well-known exotoxins. Besides the exotoxine, they also produce other metabolic products and enzymes which are known to enhance the toxicity of the toxins. It was the purpose of this work to analyse the culture filtrates for toxicity, enzymic composition and common antigens. The total and common antigens were determined using Ouchterlony's gel diffusion method. The culture filtrates were dialysed and concentrated before subjecting them to agar gel electrophoresis. The presence of the enzymes, phospholipase, phosphodiesterase, protease, nucleotidase, amino acid oxidase and esterase was established.

Production of diphtheria toxin in shaken cultures.—Studies on production of diphtheria toxin by the shaken culture method were continued using the—tryptic and papain digest of beef and veal—infusion peptone media. The strains P. W. S. 8(HI) and P. W. 8 (Wiss) of *C. diphtheria* were used for toxin production. Three hundred ml. of the medium in 3 litre Haffkine flasks was inoculated with 5.0 ml. of the culture inoculum and shaken at 80-100 revolutions per minute at 37° C for 72 to 96 hours. It was found that with the P. W. (HI) strain none of the media yielded a potent toxin. However, P. W. (Wiss) strain yielded potent toxins only with tryptic and papain digest media, the Lf of the toxins being about 50-55 I.U./ml. Further studies are being carried out varying the speed of shaking, volume of medium in the container, the amount of inoculum needed and other nutritional factors. The strain P. W. 8 (Wiss) yielded highly potent toxin of about 80 to 120 Lf in papain digest medium in stationery culture at 37 ° C. The purity of the toxoid prepared using enzyme-digested media was found to be 2 to 2 1/2 times more than the one prepared using veal-infusion peptone medium.

Studies in production of tetanus toxin.—Preliminary studies were carried out to produce high MLD tetanus toxin for the preparation of toxoid for human immunization. Two media have been taken up for study viz., (1) enzyme digest medium and (2) a semi-synthetic medium. Using the A-471 strain of *Cl. tetani*, the enzyme digested medium yielded toxin with 200,000 to 300,000 M. L. D., while using the Harvard strain of *Cl. tetani*, the semi-synthetic medium yielded toxin with 80,000 M. L. D. The toxoid prepared from these toxins are being tested for their antigenicity.

Assay of toxins and antitoxins by their cytotoxic effects in tissue culture.—Work on the assay of toxins and antitoxins in monkey kidney cell cultures was continued. Highly potent toxins prepared in enzyme-digest medium when tested in such tissue cultures gave TCD₅₀ titres agreeing closely with their M. R. D. values obtained by animal tests. Anti-diphtheritic sera collected from hyper-immunized horses were assayed in this system and the titres obtained agreed with those obtained by the routine method. The effect of gas gangrene group of toxins and snake venoms on these tissue cultures is under study. Preliminary indications are that snake venoms exert a cytopathogenic effect which is neutralised by antivenin.

Production and testing of pertussis vaccine and triple antigen.—Experimental work on the production of *H. pertussis* vaccine by shake-culture method continued. Work on the growth of *H. pertussis* in 3 litre Haffkine flasks with 300 ml. of medium on a bigger shaking machine was undertaken with a view to determine different factors such as speed of the shaker, density and volume of the inoculum, duration of shaking, etc., affecting growth. An optimum speed of 98 revolutions per minute, gave the maximum growth in 48 hours, giving a count of 35,000 to 40,000 million cells per ml. with an inoculum of 40-50 ml. seed-culture containing about 25,000 million cells ml. The inoculum with

less than 15,000 million ml. gave a final yield of the order of 15,000-20,000 million ml. after 48 hrs. A modified Cohen-Wheeler medium containing charcoal and solidified by the addition of 2 per cent. agar is being developed for the production of *H. pertussis* vaccine. A heavy growth is obtained on this medium compared to that in the usual medium without charcoal. Efforts are being made to simplify this medium to use it for large-scale production of vaccine. About 70,000 ml. of crude diphtheria toxoid prepared in Veal-infusion-proteose-peptone medium as well as in Pope-Lingood's medium and about 100,000 ml. of crude tetanus toxoid prepared in veal-infusion-bacto-peptone medium have been purified and concentrated for absorption on to aluminium phosphate gel and their final incorporation into triple vaccine. For diphtheria toxoid, the preliminary concentration of the crude antigen is affected by means of ammonium alum and the final purification by the ammonium sulphate fractionation method as described by the W. H. O. Technical Report Series No. 61 (1953). For the purification of crude tetanus toxoid, a modification of Levine's ammonium sulphate fractionation method is used and the concentration of the purified toxoid determined by the combining power method. About 25,000 ml. of triple vaccine have been prepared so far using purified and absorbed diphtheria and tetanus toxoids and concentrated *H. pertussis* vaccine. The vaccine was tested for potency of its different components in animals and the results are satisfactory. Most of the triple vaccine so far prepared has been supplied for field trials in different parts of country.

Purification and study of the anticoagulant factor in cobra venom.—From the Cobra venom, an anticoagulant was separated free from the neurotoxin and other enzymes, by the use of DEAE and CM celluloses. The anticoagulant fraction had no effect on the coagulation of purified fibrin in presence of thrombin. It was found to inhibit the production of thrombin from prothrombin. The purified anticoagulant had no proteolytic or antiproteolytic activity and was non-toxic to mice.

Studies in nucleases of snake venoms.—Phosphodiesterase of venoms of cobra and Russell's viper was identified after starch gel electrophoresis by using bis-para nitrophenyl phosphate as substrate. The enzyme was also purified from Russell's viper venom by acetone fractionation procedure. It was found to be non-toxic relatively. Preliminary experiments on the venoms of Cobra and Russell's viper show the presence of an enzyme resembling polynucleotide phosphorylase which has so far been detected in bacteria only.

Occurance of tetanus organisms in ear discharges.—Clinical materials were received from Dr. J. C. Patel of K. E. M. Hospital, Bombay for isolation and identification of *Cl. tetani*. Thirty-six samples were ear discharge swabs from cases suspected of otogenic tetanus. Ten of the 36 samples showed the presence of *Cl. tetani* in viable forms.

ROUTINE

The production of toxins and toxoids during the year under review is as follows :—

Tetanus toxin	..	..	..	918.00 litres.
Tetanus toxin converted into toxoid	..	..	..	347.00 litres.
Diphtheria toxin	..	..	..	276.50 litres.
Diphtheria toxin converted into toxoid			..	44.50 litres.
Welchii toxin	..	..	..	75.00 litres.
Oedematiens toxin	..	..	..	11.50 litres.
Septique toxin	..	..	..	66.50 litres.

The production of purified antisera during the year :—

Tetanus antisera	..	..	..	973.5 litres.
Antivenin	..	..	..	389.6 litres.
Diphtheria antisera	..	..	..	142.3 litres.
Gas gangrene antitoxin	..	..	..	202.3 litres.

Total antisera	..			1707.7 litres.
----------------	----	--	--	----------------

The number of horses under immunization at Haffkine Institute at the end of the year was 143. The number of horses, under rest and under preliminary immunization, at Pimpri was 22 and 45 respectively.

Analysis.—Fifty-six samples were received for routine bacteriological analysis. Of these, 23 were hospital clinical material, 19 were suspected food poisoning cases referred from Forensic Science Laboratory and the rest were miscellaneous samples.

8. DEPARTMENT OF NUTRITION

GENERAL

The Department of Nutrition of the Government of Maharashtra continued to be located at the Haffkine Institute and is actively collaborating in the academic and research activities of the Institute. Three research enquirie

sponsored by the Indian Council of Medical Research, the Indian Council of Agricultural Research, and the Council of Scientific and Industrial Research, are currently undertaken in the Department.

RESEARCH

Nutritional evaluation of food stuffs commonly consumed in the State.—The work consists of the analysis of foodstuffs for proximate principles, the newer B-vitamins and minerals. In all, 24 different foodstuffs, viz., red gram (without outer husk); water-cress; lettuce leaves (tender); lettuce leaves (mature); spinach; bengal gram leaves; gogu (ambadi); carrot leaves; colocasia stems; broad beans; gingelly seeds; groundnut, roasted; linseed seeds; walnuts; cloves, dry; pepper, dry; garlic, dry; beetroot; radish, white; bullock's heart; cashew fruit; raddish fruit; plantain, red variety; zizyphus; custard apple, were analysed for edible portion, moisture, fibre, carotene, thiamine, riboflavin, nicotinic acid and vitamin C. Among the foodstuffs analysed, dry cloves were found to be rich in riboflavin (600 mcg./100g.). Groundnut (roasted), linseed seeds and lettuce (tender) are rich in thiamine (489, 333, 210 mcg/100g., respectively). Groundnut (roasted) and cloves (dry) contain large amounts of nicotinic acid (14.2 and 1.2 mg/100g., respectively). Carrot leaves contain 13095 I.U. of vitamin A/100g. Cashew fruit and zizyphus are good sources of vitamin C.

Studies on the nutritive value of some leafy vegetables and their supplementary effect.—The nutritive quality of two commonly consumed leafy vegetables viz., "ambadi" (*Hibiscus cannabinus*) and "methi" (*Trigonella foenumgraecums*), were studied as supplements to jowar (*Sorghum Vulgare*). The biological values of "jowar", "ambadi" and "methi" were determined by the conventional "nitrogen balance" method, using Haffkine inbred adult male rats weighing about 175g. The biological values of ambadi, methi and jowar are 74, 83 and 79, respectively; while those of jowar + ambadi and jowar + methi are 81 and 89, respectively. The biological values of "methi" is the highest amongst the three vegetable proteins. It is 13 per cent. more in respect of jowar + methi than that of jowar alone.

The investigation on regeneration of tissue proteins was carried out in rats. The rate of regeneration of liver and serum protein was very poor in rats fed on "jowar" alone. When "jowar" was supplemented with "ambadi" and "methi" the rate increased considerably. On the sixth day liver and serum protein rose to 84 per cent. and 80 per cent. of normal values respectively, in case of ambadi, and 90 per cent. 93 per cent. of normal values respectively, in case of "methi". Although the effect on regeneration of haemoglobin and red blood corpuscles was not significant in respect of all the diets mentioned above, the diet supplemented with "ambadi" gave a slightly better response as regards the formation of red blood cells in rats.

Effect of commercial sterilization on the nutritive value of milk.—Six samples of each of boiled, pasteurised and sterilised milk were analysed for fifteen nutrients including proximate principles, major elements and vitamins, with a view to studying the alterations in chemical composition. The biological values of boiled, pasteurised and sterilised milk, determined by nitrogen balance method, using albino rats of Haffkine strain, showed that sterilized (91·6) and pasteurised milk (90·4) possess nearly the same values and are 12 per cent. more than that of boiled milk (81·2).

Studies in proteins and certain B-vitamins inter-relationships.—The study of the sparing effect of the vitamins B₆ and B₁₂ on one another and the inter-relationship of these two vitamins as far as the synthesis of protein *in vivo* is concerned was undertaken. Preliminary feeding experiments were carried out on Haffkine inbred male albino rats weighing about 35g. when they were approximately four weeks old. Twelve animals of almost identical weights were distributed equally into two groups, housed in individual cages with screen bottoms and fed *ad lib.* on two diets (1) pantothenic acid deficient basal diet, (2) basal diet plus 0·2g. per cent. Ca-pantothenate. The average gains in weight after eight weeks were 94·0g. and 145·0g. in animals fed on the basal diet and the basal diet supplemented with pantothenic acid, respectively. The average gains in body weight per day were 1·64g. and 2·60g. respectively. Besides retardation in growth, no other symptoms were seen in the animals fed on pantothenic acid deficient basal diet. After eight weeks the animals were transferred to metabolic cages and the samples of urine were collected for 72 hours. Immediately after 72 hours, collection was over, the animals in two groups were sacrificed and the livers were analysed for total pantothenic acid (free and bound) and vitamin B₁₂-1. Although a three-fold increase was observed in respect of B₁₂ content of livers in pantothenic acid deficiency, excretion of vitamin B₁₂ in urine had decreased considerably at the same time.

Essential amino acids in tissues and urine of normal and vitamin-A deficient rats.—The experiments were carried out on approximately 3-week old male rats weighing between 25 and 30g. from stock colony. In two groups they were fed on two different diets—(1) vitamin-A deficient basal diet, (2) normal control vitamin-A deficient diet plus vitamin-A supplements of 10 I.U./rat/day. Xerophthalmia was produced in all experimental animals at the end of 54 days. Three days' collection of urine of four A-deficient animals and four control animals were analysed. The excretions of leucine, isoleucine and valine in urine of deficient animals were significantly more than those of control animals. The other essential amino acids in urine are determined. Immediately after the three days' collection of urine was complete, the animals in the two groups were sacrificed, the hindleg muscles and livers dissected out, made free from connective tissues, and used for moisture, nitrogen and amino acid estimations. Leucine, isoleucine, valine, histidine,

methionine and phenylalanine contents of dry powders of hindleg muscle and liver was significantly lower in deficient animals than that in control animals.

Essential amino acids in tissues and urine in normal, hypovitaminosis and hypervitaminosis conditions.—In this set of tests, the feeding experiments were arranged in such a way that the rats in the two groups had the same average age, when the conditions of hypovitaminosis and hypervitaminosis were experimentally produced in them. The control group was also put on diet simultaneously. The detailed information on feeding trials is given below :—The lactating female rats were fed on vitamin-A deficient basal diet, 7 days after the litters were born. The young ones were weaned from the mothers when they were twenty-one days old and divided into two groups having 4 and 8 male rats in each. They were housed in individual cages and fed on two different diets—(1) vitamin-A—deficient basal diet (4 animals) (2), vitamin-A deficient diet plus vitamin-A supplements of 10 I.U./rat/day (8 animals). Xerophthalmia was produced in all the animals in group 1 at the end of 5 weeks. After a period of 4 weeks from the commencement of the experiment, 8 rats in group-2 were sub-divided into two groups of 4 animals in each. The group 2(a), normal control, was continued on the diet for a week. The group 2(b) was administered orally 60,000 I.U. vitamin-A/rat/day for a week. At the end of this period, i.e., 5 weeks from the commencement of feeding experiment, the typical hypervitaminosis A symptoms, viz. (1) dullness, (2) fracture of the hindlegs, (3) bleeding from the nose etc., were seen in the animals in the group 2(b). The urine samples of rats in hypovitaminosis, hypervitaminosis and normal conditions were collected for three days for the determination of amino acids. The analytical work is in progress. Immediately after the three days' collection of urine was over, the animals in the three groups were sacrificed and the hindleg muscles and livers were worked up for determination of amino acids.

Effect of raw autoclaved soyabean on growth, regeneration of haemoglobin and red blood cells and on levels of vitamins B_{12} in liver.—The feeding experiments were continued on the lines mentioned in the previous report, and the determinations of vitamin B_{12} in livers of rats in groups III and IV (autoclaved soyabean diet without vitamin B_{12} supplement, and autoclaved soyabean diet with vitamin B_{12} supplement.) As mentioned in the previous report, there is an appreciable difference in the vitamin B_{12} content of livers of rats fed on autoclaved soyabean diet supplemented and unsupplemented with vitamin B_{12} .

Studies in Growth Inhibitory effect of raw soyabean.—This aspect of the problem was undertaken to study whether the growth inhibition produced by feeding raw soyabean diet was of permanent nature or it could be restored to normal by feeding raw soyabean diet supplemented with vitamin B_{12} . For this purpose, 24 young rats weighing about 48g. were divided into 2 groups (1) the control group of 8 animals, (2) the experimental group of 16 rats and were fed raw soyabean diet supplemented with vitamin B_{12} , and raw soyabean diet without vitamin B_{12} supplements, respectively.

The average initial levels of haemoglobin and red blood cells counts and the average body weights in the two groups were recorded. Feeding experiments were continued for 2 months. At the end of this period 16 rats in the group II were sub-divided equally into 2 groups II-A and II-B. The group II-A was fed on raw soyabean diet supplemented with vitamin B₁₂ for 6 weeks, whereas group II-B was continued on the same diet containing raw soyabean for the same period. At the end of this period, the levels of haemoglobin and red blood cells counts and the average body weight were determined in the three groups.

The growth retardation produced by feeding raw soyabean diet for 2 months is not of a permanent nature. The supplementation of vitamin B₁₂ to the diet practically overcomes the growth depression in next six-weeks (group II-A). It also appears from the results, that there is no marked differences in the haematological picture in groups I, II-A and II-B.

The rats in the three groups I, II-A and II-B were then sacrificed and blood serum and livers were worked up for the determinations of protein and vitamin B₁₂. The analytical work is in progress.

9. DEPARTMENT OF PHARMACOLOGY

GENERAL

Five new technicians were appointed in places of those who resigned during the course of the year. Training in general pharmacological procedures, biological assays, chemical, microbiological or biochemical methods of drug testing were given to twelve candidates in one field or the other depending on the lines of specialisation. Most of these people represented pharmaceutical firms. Routine examination of 4,128 samples, which included pharmacopoeial or non-pharmacopoeial preparations showed that 949 were not of standard quality. Instances were recorded where cocaine was dispensed in place of procaine, codeine phosphate was adulterated with caffeine citrate, talc was given in place of boric acid. In many of the oral vitamin mixtures, B₁₂ content was found to be considerably less than the stated amount. Researches were carried out on drug analysis, indigenous and synthetic drugs and on some fundamental problems. Critical evaluation of the effects of various ingredients that are commonly incorporated in pharmaceutical preparations on the colorimetric assay of folic acid gave much useful information. A new biological method using isolated rabbits' auricle for the assay of digitalis and its preparations has been developed. Out of a large number of synthetic oral antidiabetic drugs that were screened, one was found to be less toxic (to animals) and more potent than tolbutamide.

RESEARCH

X. Pharmacological study of Tincture Saussurea.—Tincture Saussurea has been known for its usefulness in the treatment of chronic bronchitis and asthma. Pharmacological studies were carried out on the various fractions isolated from the root. While most of the fractions were pharmacologically inert except the alkaloidal one, the tincture prepared from the whole roots produced bronchoconstriction in the guineapig while that prepared from the defatted roots had no such effect. This bronchoconstriction seems to be due to the presence of an essential oil in the root. It is suggested that for the preparation of Tincture Saussurea, defatted roots should be used in order to derive the maximum benefit of the drug.

Pharmacological study of the alkaloids of Withania somnifers and Withania aswagandha.—The total alkaloids isolated from *Withania somnifera* (Dunal) and an extract prepared from *Withania aswagandha* (kaul) (said to be an allied species) were compared pharmacologically. They (i) produced depressor response in the cat, (ii) stimulated respiration (cat), (iii) relaxed the spasm produced by acetyl choline, histamine and barium chloride on the guinea pig's ileum and (iv) depressed the force and output of the mammalian heart. They had no sedative action in rats but prolonged the sleep induced by barbiturates. Both of them did not possess any anticonvulsant or analgesic properties in the rat.

Medicinal plants against cholera.—The screening of medicinal plants which are reported to be useful in the treatment of cholera has been continued. The new plants studied were (i) *Hollarhena antidysenteric* Wall. (Apocynaceae), (ii) *Ocinum sanctum* (Labiatae), (iii) *Allium sativum* (Labiatae.), (iv) *Blumea lacera* DC. (Compositae), (v) *Psidium guajava*, Linn (Myrtaceae), (vi) *Solanum malongana* (Solanaceae), (vii) *Poinciana pulcherina* (Leguminosae) (viii) *Fagonia cretica* (Zygophyllaceae). The extracts of the above plants prepared from different parts, did not show any beneficial effect in the treatment of experimental cholera even when the drugs were administered prior to infecting the rabbit.

Medicinal plants against other pathogens.—Extracts of nine medicinal plants were screened *in vitro* against *S. aureus*, *E. coli*., *S. typhi* and *S. dysentery*, flexner. The plants were (i) *Grewia populifolia* (leaves), (ii) *Psidium guajava*, (iii) *Allium sativum* (bulbs), (iv) *Fagonia cretica* (twigs), (v) *Hedyotis auricularia* (roots), (vi) *Capparis zeylanica* (fruits) and (vii) *Solanum melongena* (leaves). The alcoholic extracts from *Psidium guajava* and *Hedyotis auricularia* were found to possess antibacterial activity against the above organisms.

Synthetic antidiabetic drugs.—So far, about 75 synthetic antidiabetic drugs have been screened for hypoglycemic activity, out of which about six compounds have been found to possess significant hypoglycemic properties.

Biochemical studies with one of them, R-94, which was found to be a potent antidiabetic drug, have been carried out and compared with tolbutamide. In normal rabbits, the effect of the two drugs (R-94 and tolbutamide) in intravenous glucose tolerance test show no significant difference. The actions of these two drugs on glycogen metabolism in liver have also been studied. Glycogen content in liver of normal and drug (100 mg/kg) treated rats were compared. The "total" and "free" glycogen content of the liver were estimated separately. The free glycogen content in the drug treated animals showed no difference from the normal. However, the total glycogen content of drug treated rats was significantly higher than the normal values. Tolbutamide showed a slightly higher effect than R-94 on glycogen metabolism in the liver. A simple method was developed for estimation of the drug R-94 in plasma. The absorption spectrum of R-94 in 95 per cent. ethanol showed an intense maximum at 221 m μ . Plasma level of the drug R-94 and blood sugar concentration were compared with the effect of tolbutamide in rabbits. The maximum drug concentration was reached in about 5 hrs. at which time the blood sugar was the lowest. After 24 hrs. although the plasma drug concentration showed no significant difference in the two groups, blood sugar content of R-94 treated animals was significantly less than the fasting level, while in tolbutamide treated animals, the blood sugar returned practically to the fasting level. In alloxanised rats, R-94 as well as tolbutamide had some hypoglycemic effect in moderately severe diabetes, but in severe diabetes no significant effect was observed.

Studies on cholera toxin.—(a) It has already been shown that it is possible to reproduce the clinical appearance of cholera as well as certain histological and biochemical features of the diseases in infant rabbits by oral administration of cholera toxin or intra-intestinal administration of live vibrios. Or, in other words, it was indicated that the toxin produced by the vibrios was responsible for the signs and symptoms of cholera. Evidence is now provided that such a mechanism may be in operation when an individual is attacked with cholera. Bacteriologically positive as well as bacteriologically negative stools (human) were fed to infant rabbits after due processing. It was observed that the majority of the bacteriologically positive stools when fed to nine to ten days old rabbits produced typical signs and symptoms of experimental cholera. A few samples of bacteriologically negative stools also behaved in the same manner. Several samples of stools from normal persons when fed to infant rabbits produced no ill effect. (b) The preparation of cholera toxin by the method recommended by Gallut is time consuming and a costly procedure. Preliminary experiments conducted to investigate the possibility of liquid media for the production of cholera toxin gave encouraging results. The medium suggested by Col. Felsenfeld produced a more potent toxin. Studies on the stability of cholera toxin revealed that the toxin can be kept potent for as long as one month at 20° C. whereas it was destroyed at 56° C and still more rapidly at 100° C. Lyophilization helped in maintaining the potency. (c) Detailed studies on the fractionation

of cholera toxin were undertaken. Amongst the various methods employed to prepare cholera toxin only Gallut's and thymol methods have shown consistant results as regards toxicity and cholera-genic properties. Therefore these two types of toxins were prepared and fractionated into three main parts. (i) lipid, (ii) protein and (iii) polysaccharides. These fractions were fed to infant rabbits to study their cholera-genic properties. It was found that the lipid fractions played an important role in producing the disease. A number of new strains of cholera were isolated from true cholera patients. These, as well as strains isolated from water sources in Calcutta, were collected. Some of them were tested for their ability to produce experimental cholera and also for their capacity to produce potent toxin.

Cellular and humoral immunity in plague.—The importance of cellular and humoral immunities in plague infection in the monkey was studied. It was possible by three successive cross-circulation experiments between monkeys, to replace the blood of a fully immunized animal with normal blood from a non-immune animal to the extent of 87·5 per cent. so that the recipient monkey now possessed 100 per cent. cellular immunity and 12·5 per cent. humoral immunity. Similarly, by cross-circulation experiments between a non-immune monkey with three successive fully immunized monkeys, the recipient monkey could be made to have 87·5 per cent. humoral immunity with no cellular immunity. When these monkeys were challenged with a certain lethal dose of *Pasteurella pestis* (30,000 million) one could observe their behaviour either leading to death or survival with reference to the degree of cellular or humoral immunity they possessed. Monkeys having 100 per cent. cellular immunity with as little as 12·5 per cent. humoral immunity were able to withstand the certain lethal dose of 30,000 million organisms. Animals possessing 12·5 and 25 per cent. humoral immunity and with no cellular immunity exhibited a survival rate of about 33 per cent and of 50 per cent respectively following the challenge dose. Animals possessing 75 per cent. humoral immunity only or above could resist the infection fairly well. There was no correlation between the agglutinin titre of the sera and the ability of the monkey to resist plague infection.

Assay of digitalis and its preparation.—The unofficial methods of assay for digitalis and its preparations were reinvestigated. The study of the method involving the use of the isolated outer wall of the right ventricle of guinea-pig heart showed that log time to maximum amplitude might be taken as a suitable metimeter for the assay of digitalis. However, this method did not have any special advantage over the present official technique since the ventricles, like the guinea-pig, could be used to measure only a single response to a single dose. The use of the isolated rabbit's auricles has been recommended for the assay of digitalis and its preparations. The increase in amplitude was proportional to the dose of the drug added and that it was reversible. The standard and the test could be compared on the same auricles. The isolated auricles method was found to give comparable results ; it was cheap and less

time consuming. The use of cellulose powder column for the assay of digitalis was studied. It was found that from a known mixture of pure digitoxin and desacetyldigilanid-A, the recovery of the components was about 90 per cent. Therefore, digitalis leaf samples were assayed using the cellulose powder column. The chemical assays of the total glycosides and the two fractions "digitoxin" and "primary glycosides" indicated that the chemical assay using 3 : 5 dinitrobenzoic acid was not satisfactory. Whereas on the other hand biological assays of the total glycosides and the two fractions "digitoxin" and "primary glycosides" indicated that these fractions constitute the total activity of the digitalis leaf samples and that they were equal in activity. Biological assays of pure digitoxin and desacetyldigilanid-A showed that primary glycoside A was three times more potent than digitoxin.

Estimation of folic acid.—The colorimetric method for the determination of folic acid developed by Hutchings and his collaborators has been investigated in respect of its reliability in the presence of a variety of ingredients very frequently incorporated in multivitamin preparations containing folic acid. Riboflavin, ascorbic acid, nitrates, ferrous-gluconate, ferrous sulphate and ferric ammonium citrate that were found to interfere in the estimation, have been studied further to arrive at their maximum amounts which would allow a fairly satisfactory recovery of folic acid during routine estimations by the colorimetric method.

Microbiological assay of biotin.—Studies were undertaken to investigate the probable use of indigenous strains of Lactobacilli as analytical tools for the assay of biotin. Ten indigenous strains of Lactobacilli, one strain each of Leuconostoc and Streptococcus were employed for this investigation. The biotin requirements of these strains were studied both qualitatively as well as quantitatively. The following three strains viz. *L. acidophilus* 157, *L. Casei* 2090 and *L. mesenteroides* P-60 gave good responses, when compared with the standard strain *L. arabinosus* 17-5. The effect of fatty acids like oleic acid and palmitic acid on the growth of these organisms was later studied. The growth of *L. Casei* and *L. mesenteroides* was inhibited, while on the other hand the growths *L. of acidophilus*. 157 and *L. arabinosus* were not affected by as large a dose as 2000 γ /ml. of oleic acid. It was also observed that small amounts of oleic acid (10 γ /ml.) in combination with 0.5 ml of biotin exhibited slight inhibitory effect which became pronounced with the increase in the amount of oleic acid.

ROUTINE

During the year many varieties of drugs were examined. They were pharmacopoeial preparations, patent and proprietary medicines which included biological and special products, chemotherapeutic remedies, analgesics, antipyretics, hormones, spirituous medicines, antibiotics, vaccines, sera, etc. In all 4,128 samples were examined out of which 949 or about 23 per cent

were found to be of non-standard quality. The main sources from where the samples were received were the Drug Control Administration of the composite State of Bombay and later, the States of Maharashtra and Gujarat. Facilities for testing have been continued for the State of Gujarat for a period of five years. Drugs were also received from the Departments of Police and Prohibition. Limited facilities were given to commercial firms for testing of their samples and this too for those drugs which could not easily be tested elsewhere. The number of samples tested under the Drugs Act and the Rules thereunder were 2,212 as against 2,118 of the preceding year. However, the percentage of drugs which were found to be of non-standard quality was 25 which was less than in the previous year, thereby suggesting a slight improvement in the quality. A substantial portion of the samples tested belonged to the category of F Schedule C and C1 (Drug Rules), the number being 2,020 out of a total of 4,128.

Pharmacology Section.—In India, Digitalis is grown mainly in the northern parts of the country. Attempts are also being made to cultivate them in the South. Digitoxins isolated from such plants, was it tested for their biological activity. Five samples examined, showed potencies of 600 to 1,200 I. U. of Digitalis per gram. Rigid control is necessary for parenteral preparations of liver extracts so that they do not contain depressor substances more than the limits prescribed for them. In all, 65 samples were tested. The range of total depressor substances contained in them varied from 0.01 to 0.21 mg. per ml. Toxicity tests were performed on a variety of substances. They totalled about 87 samples and consisted of synthetic organic compounds, antibiotics, biological products, etc. Tests for sterility and for the absence of pyrogens continued to be in heavy demand. One thousand and fifty-four samples were tested for sterility and 909 for pyrogen. The number of samples that did not pass the-test did not exceed 5 in both the cases. Amongst the usual substances that were tested for pyrogen, mention may be made of calcium gluconate, water for injection, normal saline, casein hydrolysate, phosphorus 32, non-radio active colloidal gold, and antibiotics. In all, 21 samples of insulin were tested biologically. All of them were found to be of standard quality. Testing of antitoxins and vaccines received greater attention than during previous years. Fourteen samples of tetanus antitoxins, one of diphtheria antitoxin, 2 of triple antigens, 4 of vaccines were tested. All of them were found to be of good quality. Samples of contraceptives are now being referred to us for testing. They may be broadly divided into two classes, namely, those that act locally and those that act orally. One of the later group of substances was tested on rats. The results were not encouraging. The department was frequently called upon to test indigenous remedies against poisonous snake bites. Some claims were so unscientific that they deserved hardly any consideration. Five indigenous drugs were tested in dogs injected with snake venom. None of those remedies gave any indication of their usefulness.

Biochemical Sections.—Amongst the variety of drugs that were dealt with by this section, the important ones were vitamin preparations, mostly of a complex nature, certain enzymes and combination of antibiotics. Out of the 900 samples tested, about 35 per cent were not of standard quality. It is well known that certain vitamins like vitamin B₁₂ and vitamin A, pantothenic acid are difficult to stabilize in oral mixtures when dispensed in combination with other vitamins. This has already been pointed out. To this list of vitamins may now be added vitamin B₁₂ too which has proved to be very unstable or incompatible in those oral preparations which we had the opportunity to examine. Two hundred and fourteen samples containing vitamin A were tested. Out of these, 62 samples failed to conform to the claims made. About 20 per cent out of a total of 524 samples containing vitamin B₁, were not of standard quality. Two hundred and forty-one samples containing vitamin C were tested. About 76 per cent. of these were of standard quality. Of all the vitamin preparations that were tested here, pantothenates and folic acid appear to be the worst from the point of view of stabilization. Out of 127 pantothenates about 41 per cent were not of standard quality. Similarly, 44 per cents of the folic acid samples out of a total of 140, did not conform to the claim made. Two hundred and eighty samples containing vitamin B₁₂ were examined. Most of them were simple injections. These injectable products generally were of standard quality. Out of the 20 per cent of the samples which were not of standard quality, oral B₁₂ mixtures formed the bulk. In other words, caution should be exercised when B₁₂ is dispensed in liquid preparation meant for oral administration. Out of 33 samples of enzymes tested for proteolytic activity about 76 per cent were found to be of standard quality. During the year, microbiological methods of assay for chlorotetracycline, oxytetracycline and tetracycline were standardised. The method for the assay of neomycin was also developed. As many as 121 preparations of antibiotics were examined for potency and other pharmacopoeial tests. These include penicillin and its various salts, streptomycin and its combination with penicillin, isonicotinic acid hydrazide and sulpha drugs, chloramphenicols and tetracycline compounds. The highest percentage of the samples examined pertained to penicillin preparations (57 per cent); others were streptomycin (23 per cent), tetracyclines (12 per cent), chloramphenicol (9 per cent). In all, 9 samples failed to come up to the required standard. Out of these 9 samples, 7 belonged to the penicillin group and one each of streptomycin and tetracycline. All the chloramphenicol samples were found to be of standard quality.

Pharmaceutical Section.—The variety of drugs that were tested were galenicals, essential oils, sulpha drugs, hormones, and many others which needed chemical assay. The number of spirituous medicinal preparations referred to here were appreciably less than during the previous years. Many samples turned out to be spurious. Most of the failing samples were coloured alcohol labelled as Extractum Rauwolfiae Liquidum I. P., Tr. Benzoin, B. P. or Liq.

Iodi Mitis B. P. Several popular remedies like “*Worm Powders*”, “*Skin Ointments*”, Eucalyptus Oils, Gripe Mixtures were either of substandard quality or adulterated and even a few of them were spurious. Several *Allopathic* drugs were dispensed along with *Ayurvedic* drugs and were sold in the market as the latter category of drugs. Forty-eight samples of Eucalyptus Oil were tested, out of which 12 failed to comply with the required standard for solubility and cineole contents. Most of these samples were mixed with Oil of Turpentine which could evidently be recognised by its peculiar smell. It was interesting to observe that all the samples of Eucalyptus Oil of good quality showed on optical rotation between $+5$ to $+10$. Indian Pharmacopoeia recommends an optical rotation of -5 to $+10$; this range is rather wide and is not very helpful in detecting adulteration with other volatile oils. As many as 37 samples of tincture of iodine were tested under the Drugs Act, out of these 30 (*i.e.*, 81 per cent) were found to be not of standard quality. In 6 samples, iodine was totally absent whereas in the others the content was considerably less. Ten samples of Gripe Water out of a total of 38 were found to be of substandard quality. A popular brand of Gripe Water continued to be imitated. The imitation product invariably contained saccharin instead of sugar, while sodium bicarbonate was absent. One such sample contained calcium hypophosphite while another contained calcium hypophosphite and magnesium sulphate. One sample labelled to contain sodium phosphate, on analysis, failed to show the presence of phosphate. Three samples of iodoform contained no iodoform; instead, two contained yellow coloured boric acid, whilst the third contained sulphur. One sample of codeine phosphate was adulterated to the extent of 88.4 per cent with caffeine citrate. Two samples of boric acid were found to contain talc instead. One sample of zinc oxide contained 39.18 per cent of kaolin. Two unlabelled samples of pain reliever showed the presence of aspirin, phenacetin and salicylic acid. A number of samples of pain balm having no formulae or labelled as *Ayurvedic* drugs showed the presence of phenolic compounds and soft paraffin. One sample of “*Worm Powder*” without any formula, contained santonin whereas another did not show the presence of santonin or sodium bicarbonate even though they were labelled to contain the same; another sample contained only talc. Three samples of “*Kadhas*” (infusion) were tested which did not display any formulae. On analysis they were found to contain magnesium sulphate. One sample of *Ayurvedic* analgesic mixture contained sodium salicylate and magnesium sulphate. A sample of “*Kriminashak*” contained ammonium alum. Two samples of eye remedies contained zinc oxide even though this was not disclosed on the label. A majority of “*skin ointments*” contained boric acid, zinc oxide, sulphur, salicylic acid and paraffin even though these were not mentioned on the label. One drug dispensed as “*Injection of Procaine Hydrochloride 2 per cent*”, on examination was found to contain cocaine hydrochloride to the extent of 4 mg. per ml.

Pharmacognosy Section.—Out of a total of 123 samples examined, about 77 per cent were of substandard quality. The bulk of these (32) were samples of “Ganja” which were probably stored for a long period with the result that they had deteriorated and were unfit for human medication. Eleven samples of absorbent cotton wool out of a total of 26, were of substandard quality and mostly failed in the absorbency test. It has been observed that certain manufacturers use optical brighteners for preparing surgical dressings. This practice should be discontinued. It is suggested, therefore, that I. P. should incorporate into the respective monographs a test for the absence of fluorescent substances. Out of 21 samples of other surgical dressing like Lint, Gauze, and Bandages, 12 were note of standard quality, mostly not complying with the requirements of threads per inch, warp and weft, and absorbancy. Eight samples of crude vegetable drugs were examined. All of them except one were of good quality.

10. DEPARTMENT OF VIROLOGY

GENERAL

There were no administrative changes in the department. Dr. Mariam George, Ph.D., was posted to the department as a pool officer to the C. S. I. R. in December 1960. There are two students working for the Ph.D. and two for the M.Sc. degrees.

RESEARCH

Immunogenicity of influenza vaccines and standardization of testing procedure.—Work on standardization and assay of potency of influenza vaccines was continued. A mouse lung adapted strain of Asian virus is being maintained in the laboratory. On serial passage the strain does not show increased virulence for mice. Different lots of vaccines prepared at the Institute were tested for antigenicity in mice by single dose and multiple dose immunization followed by direct challenge using this strain. Influenza vaccine was prepared in small quantities from time to time, using the locally isolated strain A/India/47/57 of the Asian type. The vaccine was tested for hemagglutination titre, antihemagglutinin production in mice, production of virus neutralizing antibodies in mice quantitated by virus neutralization tests in ovo, and for protective value by direct challenge in mice. The importance of using a strain which is similar or very closely related to the strain responsible for an outbreak is clearly demonstrated in the experiments concerned. The protective value, as tested by the direct challenge method, does not depend only on the number of hemagglutination units (HA) contained in the vaccine. Vaccine prepared and stored for a period of $2\frac{1}{2}$ years has been periodically tested; it was observed that the HA titre was a fair indication of the potency of the vaccine for a period upto $1\frac{1}{2}$ years, after which there was a sharp fall in potency

though the fall in HA titre was insignificant. The HA titre has been steadily falling since then (in a few batches upto the extent of 50 per cent.) but the protective value is only about 5 per cent. as compared to freshly prepared vaccine of the same strain and same HA titre. Vaccines prepared at the same time by two different methods and containing an equal number of HA units do not protect mice to the same extent. However, different batches showing identical HA titre, prepared by the same method, have not shown much variation in protection. It is evident that HA titre gives an indication of the amount of the virus present but all the virus contained in the preparation may not produce antibodies. HA, which is a property of the virus, does not deteriorate to the same extent as antigenicity during processing or storage. Further observations have to be made before these potency tests are applied to the manufactured product.

High titre sera have been prepared against 7 selected representative strains. Antigenic and recombination studies with selected representative strains from each type are in progress.

Adaptation of rabies street virus to chick embryo and study of changes in pathogenicity.—The two strains, 64/57 and 13/58, adapted to eggs were further serially passed in eggs for 52 and 40 passages respectively. Simultaneously, two substrains from strain 13/58, were created at egg passage 28, by separately passaging embryo head and embryo body suspensions in different batches of eggs. All these adapted strains were tested for pathogenicity for different laboratory animals at various egg passage levels. Results of such tests show that although no further change in pathogenicity of strain 13/58 has occurred, there has been a significant alteration in the progress of infection in the inoculated animals. This is apparent from the prolongation of period between the onset of symptoms to death of the animals even in case of animals inoculated intracerebrally. The death of such animals could possibly be due to starvation and dehydration rather than rabies infection as the animals were unable to either eat or drink while paralysed. The pathogenicity tests indicate slight modification in pathogenicity, however, further work will have to be carried out before any definite conclusion can be drawn.

Flury Vaccine.—Four batches of high egg passage flury vaccine were prepared, lyophilised and titrated for viral content. The passage levels and titres were (1) 207 A₂—10⁻⁴; (2) 207 L—10^{-4.5}; (3) 208A₂—10^{-5.4}; and (4) 208 L—10⁻⁵. The potency tests of these 4 batches of vaccines were carried out in guinea pigs, according to the recommendation of W. H. O. and found satisfactory. Arrangements have been made for field trials which will continue for a period of 1 to 3 years.

Adenovirus.—In the current year, 9 more cytopathic agents have been isolated bringing the total to 17. Of these, 2 were isolated from tonsils, and are not yet identified. They do not belong to the adenovirus group. Two strains were

isolated from nasal swabs of 2 patients with acute pharyngitis. A total of 20 nasal swabs and 35 throat swabs were tested. Twenty per cent. of these patients showed a fourfold or greater increase of c.f. antibody in their convalescent sera. Five isolations were made from the conjunctival swabs of 45 persons with clinical syndromes such as acute conjunctivitis (purulent and nonpurulent) and trachoma. One of these, isolated from a trachoma case, has been identified as adenovirus type 11. The others gave positive complement fixation with adenovirus antisera and are yet to be typed. Growth characteristics of 4 adenovirus strains isolated here and the cytological pattern of the host cells (monkey kidney) at different stages of their multiplication have also been studied. Collection of clinical samples and attempts at isolation of viruses are in progress. The tissue cultures that are prepared and regularly being used are (1) Hela, (2) monkey kidney and (3) human amnion; in addition, hamster kidney cells and chick embryo fibroblast cultures are prepared as and when required.

Study of Kyasanur Forest Disease (KFD) virus strains and preparation of a suitable vaccine—

The following stocks were prepared :—

(a) Virus suspensions of strains P9605, G11338 and KF37, KF34 and KF31 (recent human strains).

(b) Antigens for HA and CF tests by acetone ether extraction from P9605, G11338, KF37 and KF34.

(c) Hyperimmune mouse serum against P9605.

Serial passage of strains in different host systems was made as follows :—

Strain.				Mice.	Eggs.	Tissue culture.
P9605	..	..	..	15	21	5
G11338		..	..	24	27	5
KF37	..	..	..	19	15	5
KF31	..	..	..	15	15	

Titration of strains at different passage levels from different host systems was made in Swiss albino mice. Mouse brain virus consistently gave LD 50 titres around 10^{-9} while egg and tissue culture material titres were about 2 log doses lower. A larger number of tests is necessary before drawing any conclusions.

RSSE vaccination of department personnel was done with RSSE formalin inactivated mouse brain vaccine received from Washington; 19 persons received 3 doses each and 5 received 2 doses. Blood samples were collected before each dose and after vaccination.

14 sets of sera from vaccinated persons were tested for HI antibodies to KFD; 8 showed antibodies in the pre-inoculation sample, and two of these gave a history KFD infection. Since common antigens among Group B arbor viruses exist, the other positives were presumably due to infections of other Group B viruses. One person showed development of H. I. antibodies in the post vaccinated sera. An incidental Group B infection cannot be excluded and is being further investigated. The RSSE vaccine used appears to be ineffective for production of antibodies against KFD.

Vaccine : Formalin inactivated vaccines were prepared from strains P9605, G11338, and KF 37 at early mouse passage levels. A single potency test has been performed. Two I. P. injection of vaccine on 0, and 3rd day were followed by I. P. challenge on the 7th day. The three KFD vaccine show a protective activity of about 5 logs ; the protective activity of the RSSE vaccine was 1.6 logs.

Tissue culture of trypsinised whole chick embryo are being prepared and passage of selected strains in this system undertaken.

Polio myelitis—Serological studies.—433 sera were tested for the presence of antibodies against the three types of poliovirus. Of these, 51 were tested quantitatively and 382 qualitatively. Nine batches of monovalent polio vaccine (Salk type) were prepared and tested for safety and potency. All the batches were found to be satisfactory. One batch of trivalent inactivated vaccine has been prepared and tested. Further work on the preparation of the inactivated vaccine has been stopped. Preparation of high titre type specific antisera against polioviruses is continued ; thirty-six rabbits are immunized against the three types of poliovirus.

Preparation of typespecific antisera against ECHO and Cocksackie Viruses.—One hundred and twenty-six rabbits have been immunized against 18 ECHO strains and three Cocksackie viruses. Antisera against 14 ECHO viruses and 3 Cocksackie viruses have been prepared and tested. All sera except those against ECHO type 14 and ECHO type 25 have been found to be satisfactory. Antisera against ECHO types 8, 9, 15 and 20 are being tested.

Nagpur Encephalitis.—During the early monsoon of 1958, Nagpur was affected by an epidemic which predominantly attacked children, caused encephalitis—like symptoms and had a high mortality rate. Different aspects of the disease were studied, but without throwing much light on its ethiology. More than one of the enteric viruses were isolated from stool samples and throat swabs by investigators from the Pasteur Institute, Coonoor, and Virus Research Centre, Poona, but since there was not sufficient serological evidence and different viruses were isolated, the cause of the disease could not be pinned down to any particular virus. During the summer and monsoon months of 1960, the cases occurring at Nagpur were studied. Through the kind co-operation of the authorities and staff of the Nagpur Medical College and Hospital, it was possible to study 38 cases, clinically diagnosed as encephalitis and to

collect specimens from the same. Specimens have also been collected from 8 cases in Bombay through the kind co-operation of the J. J. Hospital and Wadia Children's Hospital. In all there are 39 cerebrospinal fluids, 43 rectal swabs and 38 throat swabs being studied from the above mentioned cases in Monkey kidney tissue cultures and suckling mice. For serological studies 14 acute and 3 convalescent blood samples could be obtained. So far one cytopathic agent has been isolated in monkey kidney tissue culture from the CSF sample of a fatal case that occurred in Nagpur. Concurrent studies on encephalitis in children in Bombay are also carried out for comparison, and one cytopathic agent has been isolated from the rectal swab of a child, showing paralytic sequelae of fever at the J. J. Hospital Bombay. These isolates are being identified and further investigations of the clinical materials are being continued.

ROUTINE

Supply of Biologicals.—*Antirabic vaccine.* During the year 4,373,246 ml. of vaccine were supplied as compared to 3,613,057 ml. in 1959. No fresh indent for high titre antirabic serum was received from treatment centres.

Yellow Fever Section.—This section has been shifted to the Seamen's clinic since October 1960.

Frei's antigen.—Preparation of the antigen was continued. Samples were supplied to various hospitals for clinical trials and report.

Diagnostic.—76 brain specimens were received for rabies diagnosis. Nine were from human autopsies and the remaining were animal brains. All human brains were negative. Of 67 animal brains examined, 26 dog brains were positive; 9 dog salivary glands were found to be positive by biological test in mice.

11. BLOOD BANK SECTION

GENERAL

The demands for dried human plasma and the blood grouping sera were on increase during the year and it was not possible to meet them due to insufficient number of blood donors. To secure more blood donors, the mobile blood collecting team visited certain city offices and colleges. The department took part in city exhibitions, by putting up its own stall. All along these years, the response from the voluntary blood donors was negligible. However, in this year, 178 persons donated blood voluntarily and also 200 members of the staff of the Institute. Two batches of candidates have completed their training in this section. In addition, sixteen laboratory technicians were trained in Blood Bank methodology.

RESEARCH

The preparation of Rh Anti-D serum.—The Rh negative blood donors are being immunized with Rh positive blood. So far 6 donors have responded by forming anti-D antibody. These donors have also developed anti-C antibody.

Hence, further immunization has been stopped for the time being. Efforts are being made to absorb anti-C antibodies from the sera collected so far from these donors.

Rh grouping of blood donors.—The study of Rh blood groups and their subtypes amongst the blood donors was carried out. During the year, 1500 blood donors were grouped for Rh factor and Rh sub typing was carried out in 176 blood donors.

Preparation of Anti-A and Anti-B serum.—There is an increasing demand for these sera from all over the country. The source of these sera are blood donors. But the sera of these donors have low titres of antibody and avidity. These titres can be raised by immunizing these donors with specific group substances. These substances were prepared from meconium by chemical extraction. The volunteers who were immunized with these substances responded satisfactorily. In this way, we were able to meet the increasing demands for these sera.

The standardisation of anti-A and anti-B grouping sera.—The sera prepared by the Haffkine Institute compared favourably with the W. H. O. international standards.

Plasma containing Anti-haemophilic globulin factor.—Fifty bottles of such plasma were prepared. It was reported that patients who received this plasma responded satisfactorily.

Preservation of tissues from cadavers.—Preliminary methods with animal tissues were continued during the year. The blood vessels, of dogs were dissected under sterile conditions. Next they were frozen and dried. Experiments are being carried out to put back these preserved vessels into dogs.

Preparation of plasma fractions from placentas.—Further batches of globulins were prepared from placental blood by ammonium sulphate precipitation methods.

ROUTINE

During the year 3784 blood donors were bled, for the preparation of dried plasma. From this 483·750 litres of dried plasma were prepared. One hundred and one litres of dried plasma were distributed free and 406 litres were sold making a total of 507 litres. In addition to this, 150 litres of liquid plasma was received from the Armed Forces Medical College, Poona, for freezing and drying. The haemoglobin test, ABO blood groupings and serological tests, were performed on the blood samples of all blood donors as a routine. From the various hospitals and Medical personnel 265 samples of blood were received for Rh blood grouping, blood group isoimmunization tests, Coomb's tests etc. For the preparation of Anti-A and Anti-B grouping sera, 83,050 ml. of blood was supplied to the Clinical Pathology department. For the Department of Immunology 46983 ampoules of Antivenine were frozen and dried. The section has started carrying out paternity tests in cases of disputed parentage from this year. During the year, six such cases were investigated.

12. MEDIA SECTION

GENERAL

Twenty five short term trainees were trained for preparation of culture media, in sterilization techniques and production of parenteral injections.

RESEARCH

This department supplied special media to various departments. Enzymatic digest of casein with substantial tryptophan content for parenteral injection is being tried. Several enzymes are being used for this work.

ROUTINE

The following were prepared during the year :—

1. Casein hydrolysate for Cholera Vaccine .. 14,997·8 L.
2. T. A. B. Bottles for typhoid vaccine .. 5,624 bottles of 200 ml.
each.
3. Roux Bottles for Cholera Research in 1,662 bottles 200 ml.
Pharmacology each.
4. Sterility medium bottles 55,224 bottles 80 ml.
each.

Parental solution section.—Below are the quantities of the injectibles issued :

				Media litres
Glucose saline	..	..	..	44,057·5 litres.
5 per cent Glucose solution	..	..	..	37,356·5 „
25 per cent Glucose solution	..	..	..	2,565·7 „
50 per cent Glucose solution	..	..	..	78·4 „
Normal saline	..	..	..	9,257·0 „
Hypertonic saline	..	..	..	2,447·0 „
Protein Hydrolysate	..	..	..	451·6 „
Sodium sulphate	..	..	..	95·0 „
Sodium citrate	..	..	..	463·0 „
Anti-coagulation solution	..	..	..	2,737·0 „
Distilled water	..	..	..	862·0 „
				100,270·7 litres.

13. ANIMAL HOUSE

GENERAL

The production and supply of laboratory animals for experimental purposes has shown a steady increase and in more than that of the previous year.

Training.—Students of “Technician Training Course” were given general instruction in breeding, feeding, handling and general management of various laboratory animals. Dr. K. L. Rao from Rajasthan Veterinary College, Bikaner, joined the section on 22nd October 1960 for 3 months of training in breeding, and general management of laboratory animals, etc. The following tables show the number of animals bred, issued to the Institute, sold to other laboratories and purchased during the year 1960.

Species of animals.			Number of animals bred.	Number of animals issued.	Number of animals sold.	Number of animals purchased.
1.	White mice H. I.		16,900	12,398	280	525
2.	Swiss mice		40,304	23,658	434	..
3.	Rats ..		4,893	3,114	1,052	..
4.	Rabbits ..		2,115	1,490	175	..
5.	Guinea pigs		2,104	1,552	488	..
6.	Hamsters ..		244	15	9	..
7.	Cotton rats		108		6	..
8.	DBA (old)		350	150		..
9.	C. 57 (Black)		506	303		..

Besides these animals mentioned above, the following animals were maintained in Animal House and issued for Research, tests and production.

1	Sheep			3,254	
2	Dogs			12	
3	Cats			167	
4	Monkeys			222	(Including animals maintained for Polio Research Unit of the I. C. M. R., J. J. Hospital).
5	Fowls			275	
6	Pigeon			17	
7	Horses			145	(Average number maintained for the production of different antitoxin sera).

Additional Staff numbering 16 have been sanctioned for the animal house. This includes to technicians and one tinsmith. Experiments for increasing the production of animals especially mice have been undertaken. Experiments to evolve a better and less expensive type of food have also been carried out.

14. MAINTENANCE ENGINEERING SECTION

This year "Desivac Plant" was overhauled twice. This plant has been used almost every week for freeze-drying products of other firms, blood plasma of military, as well as Institute's blood plasma and antivenine, which resulted in more work of running and maintenance of this machine. The number of charges taken out this year is about 79. This section has helped all the departments in designing small appliances, gadgets, etc. and some patterns for casting purposes. The Carpentry Unit carried out the preparation of varieties of crates and small furniture requirements of all the departments and the maintenance of all furniture and research equipment. Further advice is given to business management section and stores in scrutinizing of technical quotations purchase of machinery, research equipment and raw materials. During this year, arc welding and gas welding sets have been procured and put to commission. Plant machinery and research equipment which have been imported and purchased locally were tested, installed and put to commission. The maintenance of all these rests with this section. The section also deals with the maintenance of Institute's vehicles.

15. LIBRARY SECTION

GENERAL

This year five meetings of the Library Committee were held and reorganization and other related matters were discussed. The members of the Library Committee made useful suggestions from time to time to improve the library services. About 12 students attending the course in librarianship elsewhere were allowed to work in the Library to get a practical training in routine library work. The library staff participated in delivering the lectures for Technician's Training Course, for this year.

ROUTINE

Current Journals.—The need for additional Journals in specialized branches of Biochemistry, Microbiology, Pharmacology and Pathology was very much felt this year; hence about 33 new Scientific Periodicals were added along with 6 serial publications from Annual Review, Year Books, Recent Advances series, etc. Efforts are made to get the back volumes of these newly started journals for the minimum period of 5 years. About 12 journals in the existing subscription list were discontinued from 1961. The publishers of the Indian Scientific and Medical Journals, are sending their publications to us in exchange/gratis for indexing them in our weekly Library Bulletin. We are also getting some Journals, from foreign Institutions in exchange of our Annual Report, which is distributed to the Scientific and Medical Institutions in India and abroad.

Books.—We have added about 507 books in the Library including the books received as gratis. It is worth mentioning that we are getting all the publications of Ciba Pharma Private Ltd., from time to time as they are published.

Library Bulletin.—The weekly Library Bulletin Service is being continued and Government Sanction is awaited for the sanction of the post of clerk for the full-time work of this type which is very useful for our staff and outside research workers also.

Reorganization work.—(1) *Cataloguing.*—Cataloguing work of 2,000 books purchased in the past have been brought up-to-date in addition to that of newly purchased books this year. (2) The Institute has participated in the exchange programme under (a) United State Book Exchange, Technical Co-operation Mission, U.S.A., (b) Medical Book Exchange, U. K., (c) UNESCO Book Exchange, Paris, (d) W. H. O. Book Exchange, Geneva. Due to this participation, we have received many back issues of the Journals which were short with us. (3) The work of listing the duplicate materials with us is under preparation. The list of Journals short with us is complete.

Library Statistics during January-December 1960—

A. Readers—		Nos.
Number of visitors in the Library, for reference only ..		850
Number of readers for loan of books		181
B. Loan of books and periodicals—		
Number of books and periodicals issued to our staff ..		2,031
Number of books and periodicals issued under Inter Library loan, to other Libraries		295
Number of books and periodicals obtained for our staff, from other Libraries		120
C. Addition to the Library Material during the year—		
Total Number of books. (Acc. No. 9102-9609) ..		507
Total Number of bound volumes of periodicals. (Acc. No. 6041-7115)		1,074
Total Number of current periodicals subscribed ..		143
Total Number of current periodicals as gratis ..		105
Total Number of current periodicals in exchange ..		30
Total Number of periodicals in the current list ..		278
Total Number of Reports of Scientific Institutions received in the Library		80

16. ACADEMIC COURSES AND TRAINING OF HEALTH PERSONNEL

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

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

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

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

- | | |
|-------------------------|--------------------------------|
| 1. Dr. H. I. Jhala. | 7. Dr. A. N. D. Nanavati. |
| 2. Dr. K. Ganapathy. | 8. Dr. (Mrs.) A. Venkataraman. |
| 3. Dr. N. K. Dutta. | 9. Shri K. G. Anantanarayanan. |
| 4. Dr. P. J. Deoras. | 10. Dr. S. S. Rao. |
| 5. Dr. C. V. Deliwalla. | 11. Dr. M. V. R. Rao. |
| 6. Dr. M. V. Sant. | 12. Dr. K. D. Kulkarni. |

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

1. Dr. H. I. Jhala, 2. Dr. N. K. Dutta, and 3. Dr. M. V. Sant. The Institute is further recognised for the various post-graduate degrees by the undermentioned Universities :—

1. The Gujarat University, 2. The M. S. University of Baroda,
3. The Poona University, 4. The Sardar Vallabhbhai Vidyapeeth.

During the period under review 48 science graduates who had their names registered at this Institute were afforded facilities for prosecuting their post-graduate studies. Their Department-wise analysis is given below :—

Department/Section.				M.Sc.	Ph.D.
Biochemistry	..	..	..	3	..
Chemotherapy	..	..	..	4	6
Clinical Pathology	..	..	..	6	..
Entomology	..	..	..	1	6
Immunology	..	..	..	4	..
Media	..	..	..	1	..
Nutrition	..	..	..	4	1
Pharmacology	..	..	..	2	3
Virology	..	..	..	5	2

The undermentioned post-graduate students obtained their **M.Sc. degree** during the year :—

- | | |
|----------------------------|-----------------------------------|
| 1. Shri S. E. Mhasalkar .. | .. Chemotherapy Department. |
| 2. Kumari M. C. Patel .. | .. Clinical Pathology Department. |
| 3. Kumari B. A. Ver .. | .. Virology Department. |
| 4. Shri K. M. Khemani .. | .. Virology Department. |
| 5. Shri R. T. Talwalkar .. | .. Nutrition Department. |
| 6. Kumari Vedaprakash .. | .. Virology Department. |

Following is the list of 56 persons to whom training was given in various departments during the year :—

Department of Bacteriology

Shri M. S. Karkhanis, Bombay Veterinary College, Bombay, Shri N. G. Mehta, Shri M. S. Mayadeo, Shri J. P. Sarin, Senior Medical Officer, Provincial Hyg. Institute, Lucknow, Shri P. K. Jain, Deputy Superintendent Government Vaccine Institute; Namkum (Ranchi).

Biochemistry Department

Shri P. George Kutty Alex, Bombay Veterinary College, Bombay, Shri C. J. John, Shri Mohmed Arifuddin, Shri S. B. Joshi, Shri M. Raman, Kumari P. D. Gore, Shri G. D. Nadkarni, and Shri Gulam Haider.

Blood Bank Section

Kumari R. Varghese, Shri M. S. Mayadeo and Shri Mohamed Amin Khan, Laboratory Technician, C/o Director of Public Health Services, Jammu-Kashmir.

Department of Chemotherapy

Kumari S. B. Paranjpe, Shri G. C. Shonkeen, Shri K. H. Iyer, Shri C. J. Fernandes, Kumari R. Varghese, Shri R. S. Pagnis, Kumari K. P. Merchant, Shri J. S. Rijhsinghani, Kumari B. Kanakalakshmi, Kumari Shantakumari Nair, Kumari P. M. Choithani, and Shri P. D. Trivedi, Laboratory Assistant, Tata Chemicals, Ltd., Bombay.

Department of Clinical Pathology and Diagnostic Reagents

Shri C. R. Bhat, Kumari Ira Ray, Shri C. S. G. Rao, Senior Laboratory Assistant, Central P. H. R. I., Nagpur, Kumari Dasgupta, Kumari D. P. Bhatt, Kumari A. S. Lele and Kumari U. S. Redkar.

Department of Entomology

Shri S. J. Varghese, Shri H. S. Rama Rao, Junior Technical Assistant Central Silk Board, Bombay and Shri V. M. Shah.

Department of Immunology

Shri S. R. Koppikar, Shri A. G. Purohit and Shri S. G. Thatte.

Media Section

Shri K. S. Krishnamurthi, Shri V. S. Deo, Superintendent, Bacterial Vaccine Institute of Veterinary Biological Products, and Shri M. W. Gokhale, Research Assistant, Bacterial Vaccine Institute of Veterinary Biological Products.

Department of Pharmacology

Shri H. N. Parikh, Analytical Chemist, The Gujarat Pharmaceutical and Chemical Works, Shri N. V. Phadke, Shri Harbhajan Singh, Demonstrator in Pharmacology, Punjab Veterinary College, Hissar, Shri Y. J. Prabhu, Indo Remedies Ltd. Bombay, Shri Rajendrakumar Gupta, Khandelwal Laboratories, Bombay, Shri S. B. Gandhi, Chemist, Mac Laboratories and Shri V. K. Kumaran, Senior Chemist, Indo Pharmaceutical Works, Bombay. Shri N. S. Patel, M/s. Usan laboratories, Bombay-4.

Department of Virology

Shri D. S. Gorhe, Bombay Veterinary College, Bombay, Shri V. K. Rao, and Shri S. R. Nadgir, Superintendent Virus Vaccine Institute of Veterinary Biological Products, Poona.

Animal House

Shri K. L. Rao, Faculty Member, Rajasthan College of Veterinary Science and Animal Husbandry.

LABORATORY TECHNICIANS' TRAINING COURSE

Eighteen science graduates were admitted in the second batch of this training course for the year 1959-60, and the undermentioned 17 candidates who completed their training successfully were awarded the certificates. Shri R. C. Chaumal, Shri R. C. Desai, Shri H. K. Soni, Shri M. B. Borkar, Shri S. M. Mandpe, Kumari P. S. Awar, Shri C. S. Kashyap, Shri A. A. Khan, Shri Aliahamed A. Khan, Shri H. H. Maniar, Shri R. B. Desai, Shri R. M. Desai, Shri D. G. Dhakkan, Shri S. N. Joglekar, Shri M. B. Khare, Shri B. N. Khare, and Shri A. P. Phatak.

A third batch of 16 graduates has started training for the current session from 25th July 1960.

17. GRANTS

(a) THE FOLLOWING RESEARCH FELLOWSHIPS AND SCHOLARSHIPS WERE ATTACHED TO THE INSTITUTE
DURING THE PERIOD UNDER REVIEW

Serial No.	Sponsors.	Subject of Fellowship/Scholarship.	Name of the Candidate and period.	Amount.
1	Council of Scientific and Industrial Research, New Delhi.	For carrying out studies on Hormones and Glandular Products.	Dr. S. A. Vasavada, B.Sc., (Hons.) B.Sc. (Tech.) Ph.D., from 1st June 1959 (Senior Research Fellow.)	400 per mensem.
2	Do.	do. .. Do. do. ..	Shri P. A. Malik, M.Sc., from 5th June 1959 (Junior Research Fellow).	250 per mensem.
3	Do.	do. .. For carrying out research on "Cofactors in Protein Biosynthesis".	Mrs. Ranjan Bhatt	400 per mensem.
4	Do.	do. .. For "Studies on Proteins and certain B. Vitamins Inter-relationship". (Nutrition).	Kumari Afzal Murid	250 per mensem.
5	National Research Fellowship	.. Studies in the Nucleases in the Venoms of Cobra and Russell's Viper.	Dr. R. W. P. Master, M.Sc., Ph.D. from 1st June 1960.	400 per mensem.
6	Government of India Research Training Scholarship.	For carrying out research in Organic Chemistry.	Shri B. S. Rathore, M.Sc., from 9th October 1959.	200 per mensem.
7	Do.	do. .. For carrying out Research in Microbiology.	Shri G. P. Kamat, M.Sc., from March 1960.	200 per mensem.

8	Government of India Research Training Scholarship.	..	For carrying out Research in Biochemistry.	Kumari S. S. Fatharpekar, M.Sc., from 15th November 1960.	200 per mensem.
9	Government of Maharashtra Research Training Scholarship.		For studies in anticoagulant Action of Cobra Venom.	Kumari V. N. Wankar, B.Sc., from 1st July 1959.	150 per mensem.
10	Government of Maharashtra Research Training Scholarship.		For carrying out researches in Organic Chemistry.	Kumari S. J. Shahane, M.Sc., from 5th August 1959 to 13th November 1960.	150 per mensem.
11	Do.	do	.. For carrying out Virus Survey on Encephalitis.	Kumari Rayna Mendes, M.Sc., from 11th November 1959.	250 per mensem.
12	Unichem Laboratories, Bombay	..	For Research on "Indigenous Drugs".	Shri N.B. Oza, M.Sc., from 1st July 1960.	250 per mensem.
13	Do.	do.	.. For Research on "Pharmacology".	Kumari S. Vasantha, M.Sc., from 23rd September 1960.	250 per mensem.
14	Haffkine Institute Diamond Jubilee Celebrations Committee, Bombay.		For carrying out Research in Department of Chemotherapy.	Shri S. G. Panjabi, M.Sc., from 25th November 1959 to 20th October 1960.	250 per mensem.
				Shri D. V. Mehta, B.Sc., from 9th November 1960.	150 per mensem.
15	Do.	do.	.. For carrying out Research in Synthetic Drugs.		250 per mensem.
16	Do. (Sponsored by M/s. Dumex Ltd.)	do.	.. For carrying out Research Work in Bacteriology.	Shri M. V. Panse, M.Sc., from 10th January 1959.	250 per mensem.
17	Colombo Plan Fellowship	..	For carrying out Research Work in Bacteriology.	Shri P. M. Adhikary, B.Sc., from 6th June 1959.	175 per mensem.

(b) INDIAN COUNCIL OF MEDICAL RESEARCH, NEW DELHI.		Grants. Rs.
(i) Drug Research Unit under Dr. N. K. Dutta ..		12,640
(ii) Enquiry on the Adaptation of Rabies Street Virus collected locally to chick embryo under Dr. A. N. D. Nanavati		6,505
(iii) Enquiry on the Nutritive Value of Foodstuffs under Dr. M. V. Radhakrishna Rao		23,035
(iv) Project for the manufacture of Virus Vaccine against Kyasanur Forest Disease		1,65,000
(v) Enquiry on the preparation of type specific Antisera against Cocksackie and ECHO Viruses, under Dr. A. N. D. Nanavati in collaboration with Dr. P. V. Gharpure		9,200
(vi) Enquiry on the Isolation of Cholera Toxin and Assessment of its value in immunity to Cholera ..		2,300

18. CRITICS CLUB

Forty-nine scientific meetings were held during the year under the auspices of the Critics Club. Eleven of these were addressed by Scientists from outside. The following is the list of speakers and the subjects :—

1. Dr. W. H. Sebrell, Prof. Amino acids and protein requirements of Public Health, Nutritional of man. Sciences, Columbia University, New York, U. S. A.
2. Kumari V. N. Waknis .. Fractionation of cobra venom and isolation of anticoagulant factor.
3. Shri H. C. Shah .. The effect of Ragi (*Eleusine coracana*) on glucose tolerance in human diabetic.
4. Dr. S. A. Vasavada .. Role of anti-diabetic drugs in "Diabetes mellitus."
5. Prof. P. Grabar, Head of The use of immunochemical methods Department of Bacterial in the study of proteins. Chemistry, Institute Pasteur, Paris.
6. Smt. N. D. Maloo. .. Laboratory diagnosis of haemolytic streptococcal infections in man, with special reference to anti-streptolysin "O" titration.

7. Dr. Peter Reeves, Guest worker. Some aspects of genetics of *Enterobacteriaceae*.
8. Shri D. V. Shintre .. The estimation of m-amino-phenol in p-aminosalicylic acid and its calcium and sodium salts.
9. Dr. K. Ganapathi .. The riddle of the macromolecules.
10. Dr. R. W. Master .. L-amino acid oxidase in venoms of cobra and Russell's viper.
11. Kumari G. C. Kothari .. Bacteriological and chemical studies on dairy products.
12. Kumari C. Vedprakash .. Study of intestinal viral flora of infantile diarrhoea cases by tissue culture technique.
13. Shri M. H. Shah .. Synthesis of some s-triazine derivatives as oral diuretic agents.
14. Dr. M. V. Sant .. Prevalence of syphilis in an urban industrial centre in Bombay as compared with other urban and rural centres.
15. Shri Y. M. Umerji .. Estimation of thymol in pharmaceutical preparations.
16. Prof. A. A. Smorodintsev. New live vaccines against poliomyelitis, influenza and measles.
Institute of Experimental Medicine *Kirovsky prospect*, Leningrad, U. S. S. R.
17. Capt. Dan. C. Cavanaugh, Role of mononuclear phagocytes in the pathogenesis of flea-borne plague.
Walter Reed Army Institute of Medical Research, Washington, D. C. Guest worker.
18. Dr. A. K. Hazra. .. Modern immunological concepts.
19. Dr. (Smt.) A. Venkataraman. A water soluble preparation of cytochrome oxidase from beef heart mitochondria.
20. Kumari N. Shah .. Isoagglutinin response in blood donors to infectious of specific group substances obtained from meconium.
21. Shri R. S. Karbhari .. Liquid medium for T. A. B. vaccine.

22. Kumari Usha K. Naik .. Pasteurization and sterilization of milk—A comparative study.
23. Dr. H. J. Mansharamani .. Epidemiology of Kyasanur forest disease.
24. Dr. K. D. Kulkarni .. Cardiac glycosides from digitalis.
25. Shri M. L. Chainani .. Newer antibiotics.
26. Dr. B. G. Modi .. Pregnancy tests; a review of modern trends in the diagnosis.
27. Kumari Surbala L. Shah .. Protein interactions in sera under the influence of heat.
28. Shri R. T. Talwalkar .. Studies in essential amino acid composition of some leafy vegetables.
29. Shri K. M. Khemani .. Adaptation of locally isolated rabies street virus strains to embryonated eggs.
30. Dr. J. R. Audy, Director, Methods in epidemiological studies.
Hooper Foundation. University
of California Medical Centre,
San Francisco.
31. Shri U. S. Durgakari .. Spectrophotometric method for the determination of manganese in chemical material.
32. Shri B. D. Sohoni .. Studies on clinically diagnosed cases of otomycoses.
33. Shri S. A. Vengsarkar .. Study of amino acid requirements of locally isolated strains of lactobacilli.
34. Capt. Dan. C. Cavanaugh, Studies in Bombay rats.
Guest worker. Walter Reed
Army Institute of Medical
Research, Washington, D.C.
35. Dr. P. M. Bhargava, Head of Protein biosynthesis.
department of Biochemistry,
Regional Research Laboratory,
Hyderabad Division

36. Dr. (Smt.) A. Venkataraman Disorders of amino acid metabolism
in protein deficiencies.
37. Dr. S. S. Sabnis .. Blood groups and diseases.
38. Dr. Charles Thomas, Johns Organization of bacteriophage
Hopkins University, Baltimore, DNA.
Md.
39. Shri M. G. Nerurkar .. Hydrolysis of casein.
40. Dr. K. V. Kulkarni .. Blood Banking and plasma process-
ing in U. K.
41. Dr. K. Ganapathi .. Some regulatory mechanisms in
living systems.
42. Dr. A. N. D. Nanavati .. Rabies problem in India.
43. Shri M. L. Kakade .. Studies in food yeast.
44. Dr. S. P. W. Winteringham, Mechanism of resistance of insects
Biochemistry department, Pest to insecticides.
infestation laboratory, Slough,
England.
45. Dr. J. G. Bhatt .. Assay of Digitalis.
46. Prof. Frank J. Fenner, F.R.S. Genetics of viruses : Mutation
Professor of Microbiology John recombination and reactivation.,
Curtin School of Medical
Research, Australian National
University, Canberra.
47. Shri R. A. Bellare .. Modification in the characteristics
and growth requirements of
tubercle bacilli under the influence
of drug or drug therapy.
48. Dr. A. N. D. Nanavati .. Preliminary report on the outbreak
of polyneuritis in Bombay.
A syndrome clinically indistin-
guishable from tricresyl phosph-
ate poisoning.

19. BUSINESS MANAGEMENT

Staff.—During the year under review, the following staff changes took place :—

Stores Officer, Shri S. C. Merh, B.Sc., resigned his job. In his place, Shri A. T. Maniar, B.Sc., has been appointed as Stores Officer.

A senior Assistant Storekeeper, Shri J. B. Worlikar, retired after a long and devoted service.

Training.—Shri B. D. Wadekar, a member of the staff, was deputed to the Institute of Cost and Works Accountants of India, Calcutta, for a period of 4-1/2 months for training in cost accounting.

Classes were also conducted at the Institute to train the ministerial staff in the various subjects like purchase, imports, stores, Government accounts, commercial accounts, etc.

Special Activities.—This year saw the birth of a new section in the Institute, namely, that of Centralised Finished Products. Up till now, the finished products were held by the manufacturing departments. It has started taking over finished products from the various manufacturing departments of the Institute. All the manufactured products will be taken over by the Pharmacist as soon as the requisite space and facilities become available.

This year, the Institute switched over to the metric system and all the specifications and transactions are now being carried out in this system.

The important work of standardisation and simplification, both in raw materials as well as procedural forms, was taken up.

The Institute is also going in for printed Inland Pricelist, Export Pricelist and Therapeutic Index. In the production of these, efforts are being made to maintain the high standard. Also, commercial accounting system has been introduced in the Institute and costing on systematic lines has been carried out.

General.—Though, on the business side the tempo was maintained and exceeded, the staff difficulties, particularly of skilled and supervisory type, continued. The scarcity of godown space for raw materials and finished products has been felt all along. Mr. W. H. Eastman, Expert, International Labour Organisation, also stressed these factors in his report, which was submitted during this year.

Gifts of equipment.—In the course of the year, a substantial gift of scientific glassware worth about Rs. 75,000 was received from UNICEF.

Another gift of valuable equipment and rare chemicals was also made by the U. S. Army, who deputed Capt. D. C. Cavanaugh to this Institute for scientific studies by the Walter Reed Army Institute of Research, Washington, U. S. A.

FINANCIAL POSITION

OUR POSITION AS ON 31ST MARCH 1960

<i>What We Have.</i>	1959-60		1958-59	
	Rs.	Rs.	Rs.	Rs.
1. Land and Buildings ..	39,94,116		39,94,11	
2. Equipments ..	23,77,395		19,55,584	
3. Library Books ..	4,01,567		5,00,000	
4. Live Stock (excluding Gifts) ..	1,06,638		1,10,358	
5. Stocks and Stores ..	15,11,384		15,95,420	
6. Amount to be received ..	14,54,310		16,42,314	
7. Cash and other Balances ..	2,84,796	1,01,30,206	12,67,197	1,10,64,989

What We Owe.

1. Depreciation Fund ..	10,52,243		8,79,805	
2. Leave and pension Reserve ..	Nil.		3,79,097	
3. Amounts to be paid ..	5,32,657		15,18,843	
4. Miscellaneous Deposits ..	1,45,851	17,30,75	1,74,621	29,52,366
		83,99,455		81,12,623

Surplus to be distributed between Government and the Institute.

Net Surplus (after providing for all expenses).		7,89,441	8,99,549
---	--	----------	----------

What Remains.

1. Capital		76,10,014	72,13,074
------------------	--	-----------	-----------

What we earned and spent in 1959-60

<i>What We Earned.</i>	1959-60		1958-59	
	Rs.	Rs.	Rs.	Rs.
1. From selling biologicals and drugs ..	49,77,213		50,12,109	
2. From Analytical Tests ..	1,15,333		1,15,467	
3. Miscellaneous sources ..	41,036		44,133	
4. Value of closing stock ..	15,11,384	66,44,965	15,95,420	67,67,129

<i>What We Spent.</i>			1959-60		1958-59	
			Rs.	Rs.	Rs.	Rs.
1. On our employees	..	..	13,17,234		12,19,236	
2. On raw materials	..	..	17,81,063		18,19,592	
3. On other purchases and services ..	..	..	3,04,208		4,06,081	
4. On provision for depreciation etc. ..	..	..	8,57,600		6,66,056	
5. Value of opening stock ..	..	..	15,95,420	58,55,525	17,56,615	58,67,580
<i>Surplus to be distributed between Government and the Institute.</i>				..		
1. Net Surplus	..	..		7,89,441		8,99,549
<i>How the Surplus will be distributed.</i>						
1. 50 percent to the Government ..	..	..		3,94,721		4,49,775
2. 50 percent to the Institute's Development Fund.				3,94,720		4,49,774

This year, the value of exports came to Rs. 66,679 = 01 as compared to Rs. 36,881-37 for the the year 1959.

ROUTINE ACTIVITIES

				Nos.
1. No. of items on local purchase requisitions received from departments	..	..		5,434
2. No. of orders placed against import	..	..		1,524
3. No. of institutions and approved chemists served during 1960	..	..		2,696
4. No. of bills issued to customers	..	..		16,900
5. No. of parties supplying material	..	..		572
6. Physical Verifications	..	..		1,621
				(Items)
(a) Deadstock items				
(b) Finished Products	..	..		23,918
				(Items)
(c) Raw Materials	..	..		3,482
				(Items)
7. No. of indents checked	..	..		25,285
No. of receipt vouchers checked	..	..		6,385

20. LIST OF DISTINGUISHED VISITORS DURING 1960

1. His Excellency Mr. K. E. Voroshilov, President of the U. S. S. R. Moscow.
2. Dr. Frederick B. Bang, Johns Hopkins University, U. S. A.
3. Dr. Thomas McGowan, Johns Hopkins University, U. S. A.
4. Dr. F. E. Gluff, Johns Hopkins University, U. S. A.
5. Mr. Sterling Cole, Director-General, International Atomic Energy Agency, Vienna.
6. Mr. A. N. Rylov, Deputy Director-General, International Atomic Energy Agency, Vienna.
7. Mr. H. de Laboulaye, Deputy Director-General, International Atomic Energy Agency, Vienna.
8. His Excellency, Dr. V. J. Sukselainen, Prime Minister, Finland.
9. Prof. M. C. Thacker, Director-General, Council of Scientific and Industrial Research, New Delhi.
10. Prof. T. S. Rass, Professor of Ichthyology, Institute of Oceanology of Academy of Sciences of the U. S. S. R.
11. Dr. (Mrs.) Evangelin M. Macapagal, Wife of Vice-President of the Philippines.
12. Mr. and Mrs. E. E. King, Chairman, Daily Mirror, London.
13. Shri Homi J. H. Taleyarkhan, Minister for Public Health, Small Savings and Tourism, Government of Maharashtra, Bombay.
14. Dr. J. N. Audy, Director, Hooper Foundation, University of California, San Francisco, U.S.A.
15. Dr. Dobson, Head of World Health Organization Radioisotope Section, Geneva, Switzerland.
16. Dr. Jacques Trefouel, Director, Pasteur Institute of Paris, Paris, France.
17. Dr. Frank Fenner, John Curtin School of Medical Research, Australian National University, Canberra, Australia.
18. Dr. Arnold H. Beckett, Head of School of Pharmacy, Chelsea College of Science and Technology, London, U. K.
19. Prof. Kenneth Goodner, Jefferson Medical College, Professor of Microbiology, Philadelphia, U. S. A.
20. Shri D. K. Ghosh, Accountant General, Maharashtra State, Bombay.

21. Dr. (Mrs.) Mona Llewellyn-Johns, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, U. K.
22. Dr. La Cassagne, Cancer Biologist, Radium Institute, Paris.
23. Prof. A. A. Hoover, Professor of Biochemistry, University of Ceylon, Ceylon.
24. Dr. O. H. Klein, Prof. of Biochemistry, Medical Faculty Airlangga University, Surahaja, Indonesia.
25. Dr. Sanong Unakul, Department of Physiology, Bangkok, Thailand.
26. Dr. Nynt Thein, Biochemical Laboratory, Faculty of Medicine, Rangoon, Burma.
27. Dr. Sherafuddin Ansari, Department of Biochemistry, University of Kabul, Afganisthan.
28. Dr. Asri Rasad, Department of Biochemistry, Faculty of Medicine, University of Indonesia, Djakarta, Indonesia.
29. Dr. W. H. Sebrell, Professor of Public Health, Nutritional Sciences, Columbia University, New York.
30. Prof. P. Grabar, Head of Department of Bacterial Chemistry, Pasteur Institute of Paris, Paris, France.
31. Prof. A. A. Smorodinstev, Institute of Experimental Medicine, Kirovsky prospect, Leningrad, U. S. S. R.
32. Dr. Charles Thomas, Johns Hopkins University, Baltimore Md. U. S. A.
33. Dr. S. P. W. Winteringham, Chief, Biochemistry Department, Pest Infestation Laboratory, Slough, England.

21. MISCELLANEOUS

(a) *Exhibition Activities*.—With a view to educate and inform the public about the use of preventive measures (Vaccines and toxoids), therapeutic remedies (antitoxins and sera) and to stress the importance of human blood and plasma for saving life in case of accidents and emergencies, such as severe burns and surgical operations, the Institute participated in the following exhibitions :—

- (1) Health Exhibition organised by Rotary Club of Bombay at Nair Hospital.
- (2) Blood Bank and Family Planning Exhibition held at Cama Hospital, Bombay, and
- (3) International Food Festival Exhibition held at Sardar Vallabhbhai Patel Stadium Grounds, Bombay.

The enthusiastic response and the keen interest shown by the public who visited these above exhibitions clearly indicated that this mode of educating people in the modern methods of preventive medicine is very successful.

(b) *The Haffkine Institute Co-operative Credit Society Ltd.*—The Society has continued its satisfactory progress during the year. The total strength of the members is 437. The subscribed capital as on 30th June 1960 was Rs. 1,93,891·88 nP. (showing an increase of Rs. 8,270 over that of the previous year). The Society continued its function of advancing loans to its members. The loans advanced during the current year amounts to Rs. 18,85,550·53 nP. The society is registered as an agent under the existing rules for the following agencies : (1) Janata Policy under the Life Insurance Corporation of India and General Life Insurance. The Society has made a business of about Rs. 40,000 in insurance. (2) It continued its function in Small Savings Scheme. A Film show and lectures were arranged by the L. I. C. of India to encourage the members to realise importance of life insurance, small savings etc. The Society has invested in Government Securities, Bombay Municipal Debentures and the National Savings Certificates which amounts to Rs. 34,087·07 nP. The Society runs also a Grain Shop for the benefit of the members of staff and has transacted a business of Rs. 1,17,180·10 nP. by way of sales. The appropriation of profits for the year 1959-60 is as under :—

				Rs. nP.
Reserve Fund 25 per cent	..	..	..	2,682·94
Dividend Equi. Fund	..	..	..	150·00
Charity Fund	..	..	..	50·00
Dividend at 3·3/5 per cent	..	..	..	6,700·00
Bonus to Staff	..	..	..	324·00
Balance	..	..	..	824·79
				10,731·73

(c) *Welfare Activities.*—(1) Haffkine Institute Sports Club progressed satisfactorily with its increased multiple games activities. The membership increased from 205 to 300. All the Departments participated in the activities of Sports Club. The Cricket Team participated in E. Merck tournaments. Badmington, Table Tennis, Volley Ball were in full swing and received a very good response from the members. Matches were arranged for all these games. Three more trophies were instituted this year in addition to the existing ones. Many members showed particular interest for Indian games, Matches in these games were also arranged.

(2) *Welfare Centre*—(a) *Library.*—As a Welfare activity for the benefit of members of the staff about 1000 books added to the Library. The membership of this library at present is about 200. Books and magazines which are added to the library are on increase day by day. This activity is very useful to the members in developing their literary talents and the members are taking full advantage of the library.

(b) *UNICEF Skim Milk Distribution Scheme*.—Under this Scheme, skim milk distribution to 80 children in the age-group of 3-14 years and nursing mothers was continued. *Childrens' Day* and the *26th January Republic Day* were celebrated and prominence to children's games and entertainment was shown. (c) *S. S. C. Coaching Class*.—The members of Class IV employees of this Institute who could not complete their high-school education were given facilities to appear for S. S. C. Examination. The text-books, tuition fees and form-fees for S. S. C. Examination were provided for these students. Senior members of the staff of the Institute volunteered their services to conduct classes. About 17 students took advantage of this Facility.

(3) *Medical Treatment—Dispensary*.—The Institute dispensary continued to cater to the needs of the workers of the Institute and their dependent relatives, as usual. The treatment available is of the outdoor type. The Director extended money-aid for purchasing medicines to certain deserving cases during the year. The Clinical Pathology department co-operated by carrying out pathological examinations for the patients referred. The staff of the Institute and their dependent relatives who were suffering from tuberculosis were given the outdoor line of treatment at the dispensary regularly. Urgency of a suitable accommodation for housing dispensary is being keenly felt in view of its growing popularity. First-aid facilities are instituted. First-aid Boxes have been fitted up at different places in the Institute. Some batches consisting of the members of staff were given a course of first-aid training by members of staff who were specially trained in Nagpur.

(d) *Supervisory Training*.—Supervisory training has been one of the fields of additional activity during the year of report. The Institute participated in the eighteenth project of the T. W. I. Supervisory Training Programme conducted by the "Training Within Industry Centre" of the Ministry of Labour and Employment, Government of India at Bombay.

Shri D. V. Shintre, Junior Scientific Officer of the Pharmacology Department of this Institute was deputed for the training which he completed in May 1960. In addition to his work in the Pharmacology Department, Shri Shintre has been giving his services as the training officer in the supervisory training programme at this Institute.

The Training was introduced at various levels of supervisors, including the junior laboratory assistants. It is heartening to note that there is growing awareness of the need for such a training amongst the supervisors of this organisation and during group-discussions a large number of them showed productivity-consciousness. The total number of training groups conducted during the year was twenty-two and sixty supervisors completed their training four of whom later left the Institute. The number of supervisors fully trained during the year in various departments is given below :—

Pharmacology—17 ; Chemotherapy—12 ; Immunology—8 ; Bacteriology—6 ; Virology—6 ; Clinical Pathology—5 ; Entomology—1 ; Library—1 ; Business Management—1 (The total number comes to 60).

The Institute participated in the Discussion Leading Programme for Executives' and Job Safety Programme also, both of which were organised by the T. W. I. Centre, Bombay. Shri Shintre was deputed for this training. While the existing training programmes are going ahead briskly, it is proposed to take up a preliminary survey of the problems of each department for critical study and implementation of productivity techniques. Efforts are also being made to give further fillip to the entire activities through audio-visual programmes, panel discussions, conferences and seminars.

22. LIST OF PAPERS PUBLISHED IN THE YEAR 1960 FROM THIS INSTITUTE

1. Bhatt, J. G. Macdonald, A. D. Assay of digitalis. *Journal of Pharmacy and Pharmacology*, 12 ; 733-738, 1960.
2. Bundeally Amina, and Bellare, R.A. Tuberculostatic activity of capparid Mooni fruits. *Journal of Scientific and Industrial Research*, 19C ; 128-129, 1960.
3. Deliwala, C. V. A Preliminary note on the diuretic activity of 2-amino-4-metachloroaniline-1 ; 3 : 5 Traiazine. *Nature* ; 187 ; 1034, 1960.
4. Deoras, P. J. Residual effect of Pyrethrum. *Indian Journal of Pharmacy*, 22 ; 226-229, 1960.
5. Deoras, P. J. Studies on Bombay rats. A Note on the probable resistance of *B. bengalensis* to Plague. *Current Science*, 29 ; 275-276, 1960.
6. Dutta, N. K., Sastry, M. S. and Tamhane, R. G. Pharmacological actions of an alkaloidal fraction isolated from *Saussurea lappa* (Clarke). *Indian Journal of Pharmacy*, 22 ; 6-10, 1960.
7. Ganapathi, K. and Irani, R. J. Chemical pathways in carbohydrate metabolism ; *Journal of Scientific and Industrial Research*, 19A ; 9-18, 1960. (Work carried out at Hindusthan Antibiotics, Pimpri; forwarded from here).
8. Hazra, A. K. and Agnihotri, S. R. Occurrence of Toxigenic *Cl. tetani* in ear discharge. *Indian Journal of Medical Sciences*, 14 ; 197-200, 1960.
9. Irani, R. J., Ganapathi, K. Effect of carbohydrates and some carbon sources on the biosynthesis of Benzylpenicillin, by washed cells of *Penicillium Chrysogenum*. *Journal of Scientific and Industrial Research*, 19C ; 216-222, 1960. (Work carried out at Hindusthan Antibiotics, Pimpri ; forwarded from here).

10. Irani, R. J., and Ganapathi, K. Carbohydrate Constituents on the mycelium on *Penicillium chrysogenum* grown in media with different sources of carbon. *Journal of Scientific and Industrial Research*. 19C ; 207-216, 1960. (Work carried out at Hindusthan Antibiotics, Pimpri; forwarded from here).
11. Jhala H. I. "Vitamin B12 in Human tissues and sera". Agra University extension lectures Pub. by Agra University, Agra. 1960.
12. Kulkarni, K. D. ; Sabnis, S. S. ; Kulkarni, B. S. ; Chemotherapy of bacterial infections ; Synthesis of some pyrimidine, thiazoles and pyrazol derivatives. *Journal of Scientific and Industrial Research*. 19C ; 6-8, 1960.
13. Master, R. W. P. Histochemical procedures for the location of esterases after starch gel electrophoresis. *Biochemica et Biophysica Acta*. 39 ; 159-160, 1960.
14. Master, R. W. P., Bharucha, E. D. and Rao S. S. Blood sugar cholesterol and phosphorus levels in rabbits after injection of Russell's viper venom. *Indian Journal of Medical Sciences*. 14 ; 335-337, 1960.
15. Mehta, C. J. A comparison of microbiological and paper chromatographic methods for the estimation of minute amounts of free amino acids in the body fluids. *Journal of J. J. Group of Hospitals, and G. M. C. S* ; 257-262, 1960.
16. Mody, B. G. Validity of Semi-quantitative, Biological tests in the diagnosis of vesicular mole. *Journal of Obstetric and Gynaecology of India*. 10 ; 307-314, 1960.
17. Nerurkar, J. J., Joshi, R. N., Marathe, V. N., Phadke, G. S. and Bhawe, V. B. B-arylgutaconic Acid. IV. Synthesis of Crotono and valero lactones of P-Arylgutaconic and glutaric acid *J. of organic Chemistry*. 25 ; 194-195, 1960.
18. Patki, V. M. and Shirsat, M. V. Chemotherapy of intestinal infections part II. Synthesis of N-Acyl derivatives of P-Amino salicylic acid. *Journal of Scientific and Industrial Research*. 19C ; 4-6, 1960.
19. Rajadhyaksha, D. V. and Rao, S. S. Antigenic analysis of *Vibrio cholerae* and effect of heat on the antigens. *The Indian Journal of Pathology and Bacteriology*. 3 ; 36-44, 1960.
20. Sabnis, S. S. and Kulkarni, K. V. A survey for the detection of homologous serum hepatitis in the blood donors in Bombay. *Indian Journal of Medical Sciences*. 14 ; 518-521, 1960.
21. Sabnis, S. S. and Shirsat, M. V. Synthesis of P-Aminosalicic acid : Reduction of 4-N nitrosalicic acid to 4-amino salicylic acid. *Journal of Scientific and Industrial Research*. 19A ; 273-274, 1960.

22. Samant, M. S. ; Rao, S. S. and Modi, B. G. A Comparative study of Kahn, Meinicke and V. D. R. L. flocculation test for diagnosis of Syphilis. J. J. Group of Hospitals and G. M. College, 5 ; 25-32, 1960.
23. Samant, M. S. ; Rao S. S. and Modi, B. G. Sensitizing action of dyes on kahn test for syphilis. Journal of J. Group of Hospitals and G. M. College, 5 ; 107-113, 1960.
24. Shah, K. V. ; Nanavati, A. N. D. and Dutta, N. L. Outbreak of polyneuritis in Bombay with a syndrome clinically indistinguishable from tricresyl phosphate poisoning and with demonstration of tricresyl phosphate poisoning in a sample of Mustard oil. Indian Journal of Medical Sciences, 14 ; 183-189. 1960.
25. Shah, M. H., Mhasalkar, M. Y. and Deliwala, C. V. New trazine derivatives as oral diuretic agents. Journal of Scientific and Industrial Research. 19C ; 282-283, 1960.
26. Shah, M. H., Mhasalkar, M. Y. and Patki, V. M. Chemotherapy of tuberculosis, Synthesis of I-acyl and I-acyl 4-alkyl thiosemicarbazides. Journal of Scientific and Industrial Research. 19C ; 68-70, 1960.
27. Ver, B. A., Rao, S. S. and Jhala, H. I. Isolation of rabies fixed virus by starchgel electrophoresis. The Indian Journal of Pathology and Bacteriology. 3 ; 63-66, 1960.
28. Ver, B. A. ; Rao S. S. and Jhala H. I. Differential centrifugation in the purification of rabies fixed virus. Indian Journal of Pathology and Bacteriology 3 ; 82-88, 1960.

23. LIST OF PAPERS SUBMITTED FROM THE HAFKINE INSTITUTE AND WAITING FOR PUBLICATION

1. Bharucha, E. D., Joshi, R. N. and Venkataraman, A. The routine estimation of serum transminses in the clinical laboratory. Indian Journal of Medical Science (In press).
2. Bhatt, J. G. and McDonald, A. D. Study of component glycosides of Digitalis. Journal of Pharmacy and Pharmacology (In press).
3. Deoras, P. J. and Patel, K. K. Collection of Ectoparasites of Laboratory Animals. Indian Journal of Entomological study of India. (In press).
4. Dutta, N. K. and Nayak, K. P. Role of Essential oils and Allied drugs in Experimental cholera of the Rabbit. Indian Journal of Medical Research. (In press).

5. Ganapathi, K. Some control mechanisms in living systems and their molecular basis. Symposium, National Institute of Sciences of India, New Delhi-1. (In press).
6. Gorhe, D. S., Kulkarni, A. E. and Parnaik, D. T. Observation on the post-vaccination reactions in horses protected with South African horse sickness vaccine. Indian Veterinarian. (In press).
7. Habbu, M. K. The present position of plague in India. Indian Journal of Pathology and Bacteriology. (In press).
8. Kulkarni, K. D. and Shirsat, M. V. Chemistry of the thiazoles. Journal of Scientific and Industrial Research. (In press).
9. Master, R. W. P. and Rao, S. S. Protective action of heparin and E. D. T. A. against Russell's viper venom. Indian Journal of Physiology and Pharmacology. (In press).
10. Mehta, C. J. The plasma levels of free amino acids during uremia. Proceeding of the symposium on protein held in Mysore, 1960, on behalf of Council of Scientific and Industrial Research, New Delhi. (In press).
11. Patki, V. M. and Shirsat, M. V. Chemotherapy of Intestinal Infections. Part III. Modification in the method of Bratton and Marshall, for the estimation of N¹-acyl derivatives of sulphonamides in animal issues. Journal of Scientific and Industrial Research. (In press).
12. Shah, S. L. and Rao, S. S. Study of Protein interactions in serum under the influences of heat. Proceedings of the symposium in protein, held in Mysore on behalf of Council of Scientific and Industrial Research, New Delhi. (In press).
13. Vasavda, S. A ; Malik, P.A. ; Kulkarni, V. P., Anantanarayan, K. G. and Deliwala, C. V. Comparative Assay of insulin in some commercial preparations. Proceedings of all India. XIII Pharmaceutical conference at New Delhi.
14. Venkataraman, A. Studies of estimation of serum proteins. Indian Journal of Medical Science. (In press).
15. Ver, B. A., Rao, S. S. and Jhala, H. I. Antigenic analysis of rabies fixed virus by cell diffusion technique. Indian Journal of Pathology and Bacteriology (In press).
16. Waknis, V. N., Master, R. W. P. and Rao, S. S. Use of Ion-exchanges in the separation of proteins of cobra venom. (In press).

