

THE THOMAS BAKER, ALICE BAKER, AND
ELEANOR SHAW MEDICAL RESEARCH
INSTITUTE

NINETEENTH
ANNUAL
REPORT

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Medical Research Institute

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The Director's Nineteenth Annual Report

TO THE TRUSTEES

of the

THOMAS BAKER, ALICE BAKER, AND ELEANOR SHAW
MEDICAL RESEARCH INSTITUTE

Gentlemen,

During this year and with your approval, special attention has been devoted to research on biochemical and physiological problems. Dr. Fantl has continued his valuable studies on the mechanisms involved in the coagulation of blood, whilst the writer and his colleagues have been concerned with problems of carbohydrate metabolism. Mr. Lincoln, B.Sc., has recently joined the biochemical section of the Institute. During the war he was attached to the Chemical Warfare Section of the Munitions Supply Laboratories as Research Organic Chemist. At present he is engaged on problems of fat metabolism with particular reference to diabetes and it is hoped that eventually his work will link up with that of his colleagues who are engaged on the study of the problem of diabetes in man.

Whilst in England, your Chairman consulted Sir Henry Dale, Director of the Royal Institute, concerning any future appointments of senior personnel from overseas to the staff of the Institute. Sir Henry suggested that it would be a good idea to have a small Committee in England, who could advise on the capabilities of any promising applicants. Sir Henry stated that he would be perfectly willing to act on such a Committee and so would Professor Willis, of the Royal College of Surgeons of England. It was further suggested that Dr. G. S. Wilson, of the Institute of Hygiene and Dr. C. H. Kellaway should be asked to be members of this Committee.

It is inevitable that in a research institute associated with a teaching hospital, there are constant demands on the part of the Honorary Medical Staff for research on their clinical problems, and one of the greatest difficulties in this Institute has been the maintenance of a suitable balance between clinical and what may be termed basic or pure research.

Apart from its primary object of medical research, the Institute provides a routine biochemical service for the Hospital. During recent years, with the introduction of new and more

complicated routine tests, there have been more and more demands for assistance from the research personnel, and at times this has seriously threatened to interfere with the research work. The question of pure versus clinical research has been thoroughly discussed on numerous occasions with members of your Advisory Committee, and it was generally agreed that it would not be possible for the Director to conduct satisfactorily, both pure and clinical research. Admittedly clinical research is of paramount importance in a Hospital associated with teaching, but it must be realised that for clinical research to be of any practical value it must have a background of pure research. Frequently what, on the surface, might appear a simple clinical investigation may develop into a problem of a most intricate nature demanding all the resources of the research sections.

The only satisfactory solution of this position would appear to be the establishment of a clinical research unit in association with the Institute, and it is hoped that in the near future this may be accomplished. Prior to the war, many of the Honoraries came to the Institute to work out their clinical problems, and it is hoped that in the post-war years this practice will be resumed.

Although I have stressed the balancing of pure and clinical research, it must be realised that there has always been an attempt to devise research that might have a practical bearing. In particular this applies to Dr. Fantl's work on diseases involving the coagulation of blood. His preliminary studies were essentially of a theoretical nature, but now his findings have been applied to the elucidation of obscure blood diseases.

Apart from a few minor details, the new animal house has been completed and should be ready for occupancy at any moment. This new accommodation will result in considerable saving of labour, and will ensure a healthy stock of animals.

Mr. Douch has again rendered assistance to all workers by the construction of special apparatus. This has been particularly valuable during the war years, when it was impossible to obtain certain types of apparatus.

In my last report I referred to the importance of an Endowment Fund for the assurance of a continuous source of income for research work. The nucleus that has already been established has been increased by the allotment of £5,000 to the Institute by the Trustees of the Estate of the late Mrs. A. M. White.

We are greatly indebted to Kodak (A/asia.) Pty. Ltd. for the presentation of a high vacuum oil diffusion pump. This will be of great value to Dr. Fantl for his work on the separation, in

pure form, of various blood components associated with the coagulation mechanisms.

We are again indebted to The National Health and Medical Research Council for the financial support of research work, and the supply of essential laboratory apparatus.

I also wish to express great appreciation to Messrs. W. S. Kimpton and Sons for certain essential supplies. This courtesy was extended during the war years when it would otherwise have been impossible to obtain such goods.

The Library.

We acknowledge gifts of literature during the year from the following:— Commonwealth Serum Laboratories, The Connaught Laboratories, Eastman Kodak Company, Mr. Robert Fowler, F.R.C.S.; Walter and Eliza Hall Institute, Imperial Chemical Industries of Australia and New Zealand Ltd., Institute of Medical and Veterinary Science, Lister Institute of Preventive Medicine, The Mayo Clinic, Medical Research Council, London; Rockefeller Foundation, Royal College of Physicians, Edinburgh; South African Institute for Medical Research, Mr. A. J. Trinca, F.R.C.S.; U.S.S.R. Society for Cultural Relations with Foreign Countries, Editor, "The Laboratory Journal of Australasia."

We are very grateful to the Melbourne University and other Libraries for lending many Journals throughout the year.

PHYSIOLOGICAL AND BIO-CHEMICAL RESEARCH.

Studies on Blood Anti-coagulants:

The use of 3,3' ethylidene-bis-4-hydroxycoumarin, (E.D.C.), as a therapeutic and prophylactic agent for the control of thrombosis:

Since the results of preliminary work described in the previous report indicated that E.D.C. orally administered was well tolerated by volunteer patients, the Honoraries of this Hospital have co-operated in its application to suitable patients. Cases of thrombophlebitis and threatened pulmonary embolism have been treated, and it would appear that a distinctly beneficial effect ensues. In one case E.D.C. treatment was commenced after a laparotomy. 2.1 grammes of the drug were given over a period of seven days. The prothrombin activity returned to a normal level three days after the last dose. A second major operation was performed five days afterwards. No abnormal haemorrhages ensued. It was found desirable on clinical grounds to repeat the E.D.C. treatment a week after the latter operation. During nine days 2.4 grammes of E.D.C. were administered. Four days after discontinuation of its administration a normal prothrombin level was re-established.

The aim of the treatment is to establish a prothrombin level between 25 and 40 per cent. of the normal, but as great differences in response to the drug occurred, it was found necessary to carry out daily prothrombin estimations so that the dosage could be appropriately adjusted. Private practitioners have also been supplied with the drug, and in such instances the importance of frequent prothrombin estimations has been stressed. The ease of control of the blood prothrombin level with E.D.C. is in marked contrast to dicoumarol.

In recent years, other compounds influencing prothrombin levels in man and experimental animals have been described. This subject has assumed considerable practical importance, since drugs applied for various diseases have now been shown to exert side-effects on blood coagulation. Dr. Fantl in collaboration with Mrs. Piper (Miss M. Noël Rome) and Miss Nance has carried out a detailed study of the influence of the oral administration of quinine hydrochloride on the prothrombin level of rabbits and guinea-pigs, and also on the effect of the drug on the components of the blood coagulation system "in vitro." This was necessary since Pirk and Engelberg reported that quinine administered orally in therapeutic doses to man induced hypoprothrombinaemia. This statement was challenged by Quick, who was unable to find any alteration in prothrombin activity

following quinine administration. For the assessment of prothrombin Pirk and Engelberg used Russell Viper venom as a thromboplastic agent, whilst Quick used an extract of rabbit's brain. In our experiments the animals were treated with amounts of quinine ten to fifteen times greater per kilogram of body weight than that previously given to humans. The results obtained depended upon the thromboplastin used. When homologous brain was the source, no change in prothrombin could be observed in the rabbit and only a doubtful effect in guinea-pigs. On the other hand the results with Russell Viper venom indicated hypoprothrombinaemia in a high proportion of cases. However, there is good reason for believing that the observed delay in the coagulation time was not due to reduction in the prothrombin level because Russell Viper venom cannot be considered as a fully active thromboplastin preparation. Trevan and Macfarlane recognised that the addition of lipids or platelets will enhance the venom's activity. When viper venom was activated by egg lecithin, similar results to those obtained by the use of homologous brain extract were found. For these reasons it does not appear justifiable to interpret clotting times differing by only a few seconds from the normal value, as due to alterations in prothrombin activity when only Russell Viper venom is used. "In vitro" experiments indicated that quinine hydrochloride in concentrations greater than 100 mg. per cent., showed antiprothrombin activity and an antagonism between calcium ions and quinine was detectable.

The estimation of the clotting time of plasma, using thromboplastic agents and calcium ions, is a valuable test for the assessment of haemorrhagic tendencies, but it is not an indication of prothrombin concentration in a chemical sense. The so-called two-stage technique elaborated by Warner, Brinkhous and Smith allows a quantitative estimation of prothrombin. This method, however, is not suitable for general use. It was, therefore, thought that a procedure combining the expediency of the one-stage technique with a quantitative prothrombin estimation, would answer questions which the known techniques have left undecided. Prothrombin can be adsorbed by a variety of compounds. All the substances which have been found useful by previous workers for the removal of prothrombin from oxalated plasma have been examined. These preparations were tested for their ability to adsorb prothrombin by using the smallest amount which rendered oxalated plasma non-coagulable and the adsorbates were analysed for protein content. From the wide variation in protein values ranging from 7-40 mgs. per cent., it was apparent that apart from prothrombin other proteins

were adsorbed. The only exception was barium sulphate, which adsorbed between 2 and 3 mgs. per cent. protein nitrogen. Several other substances were tested, and the most efficient was found to be barium carbonate. (Forty-six estimations carried out on pooled human oxalated plasma obtained from 160 normal donors gave values for total prothrombin ranging from 1.7 to 2.7 mgs. protein nitrogen per 100 ml. oxalated plasma.) In view of the fact that barium carbonate adsorbed less protein than any other adsorbent, a closer investigation with the view of using this technique for a chemical prothrombin estimation was carried out. It now appears possible to correlate actual amounts of prothrombin estimated by a chemical method with the estimation of clotting time. Active prothrombin could be eluted from the adsorbates, and the prothrombin activity is assessed by incubating the prothrombin solutions with thromboplastin under a variety of experimental conditions. It was found that the system was active in inducing fibrin formation from purified fibrinogen solutions. From these results it is obvious that prothrombin isolated from human plasma is the only precursor of thrombin. Further the hypothesis, advanced by Quick, that prothrombin is composed of two components cannot be substantiated. However, a study of the reaction rate of thrombin formation in this synthetic system indicates that the conversion into thrombin is slow. It was found possible to accelerate thrombin formation in the above system when purified fibrinogen was replaced by plasma from which prothrombin had been completely removed by adsorption. Here maximal activity was reached after considerably shorter incubation times. The significance of this acceleration phenomenon is under investigation. The practical significance of these results is twofold: firstly, it should be possible to produce potent prothrombin from blood which can be converted into thrombin, and which is used as haemostatic agent in surgical procedures, and, secondly, since prothrombin constitutes only a very small fraction of the total plasma proteins, 99.7 per cent. of the latter are recovered from the treated plasma which further is only diluted by 5 per cent., and thus the treated plasma should be available for transfusion purposes. This would appear to be an advantage over the older procedures for prothrombin isolation in which the treated plasma is lost.

In the course of examining blood specimens for diagnostic purposes, several cases with haemorrhagic tendencies were investigated. The most interesting was the case of a female with an acquired haemorrhagic condition. Her prothrombin level was constantly greater than 100 per cent. when compared with normal controls. It could be shown that the condition was

due to an inhibitor of homologous thromboplastin. The details of the procedure which entails incubation of plasma with homologous and heterologous thromboplastin preparations, which should be useful in the diagnosis of haemophilia and allied conditions will be found in a paper by Dr. Fantl and Miss Nance, published in *The Medical Journal of Australia*.

Citric Acid in Bone Diseases:

In the last report, reference was made to the estimation of citric acid in the blood serum. The value of such determinations in the diagnosis of parathyroid tumours can be seen from the following case.

A 17-year-old patient had been suffering from bone deformities since childhood. Calcium, citric acid and alkaline phosphatase in the serum were markedly increased. A diagnosis of parathyroid tumour was made. The surgeon removed an enlarged parathyroid, which on pathological examination proved to be an adenoma. Four weeks after the operation calcium and citric acid concentrations in serum were at normal levels.

Fat Metabolism.

Mr. Lincoln has studied the anaerobic breakdown of acetoacetic acid by various organ extracts. It was found in agreement with earlier workers that the breakdown to acetone and carbon dioxide was entirely due to the amino acid concentration of the medium, and no evidence for an enzymatic reaction which occurs in some moulds could be found in mammalian tissue. Mr. Lincoln is also carrying out the enzymatic dehydrogenation of saturated fatty acids in the presence of methylene blue as hydrogen acceptor. It could be shown that dehydrogenation of palmitic and stearic acid occurs to an extent of approximately 10 per cent. The chemical degradation of the reaction products is under investigation. These studies are carried out with the idea of throwing light on the question of whether dehydrogenation is a prerequisite to fatty acid oxidation or whether we are dealing with an independent phenomenon.

Carbohydrate Metabolism.

The relationship of the pituitary gland to carbohydrate metabolism has been a subject of continuous interest to various workers in this Institute. Some time ago Dr. Ennor, in collaboration with Dr. Singer, succeeded in producing from rabbits an anti-serum which could protect partially depancreatised rats against the diabetogenic hormone of the anterior pituitary

gland. Unfortunately this work was interrupted by the war, but the results were promising and warrant further investigation. In the previous report mention was made of Mr. Nelson's work on the anti-insulin action of anterior pituitary extracts. By means of Gemmill's rats' diaphragm technique, evidence was produced to show that the pituitary-insulin antagonism was probably of a peripheral nature, in that the action of insulin in storing blood glucose as muscle glycogen was inhibited. Subsequently Cori demonstrated that hexokinase activity could be inhibited in tissue extracts by anterior pituitary extract. It was further found that this inhibition could be counteracted by insulin. Cori did not consider that insulin increased hexokinase activity in tissue extracts. In further experiments on rats' diaphragm muscle, more detailed analyses were made, and the writer and Mr. Nelson could only explain their findings on the basis that in the intact muscle insulin enhances hexokinase activity, and that this action is abolished by anterior pituitary extract.

Studies have also been made on the anti-insulin effect of posterior pituitary extract.

It is a well-known clinical phenomenon that a patient in mild insulin hypoglycaemia can be relieved by the subcutaneous administration of posterior pituitary extract. There has been considerable controversy both as to the mechanism of this phenomenon, and as to whether the vasopressor or oxytocic principles were concerned. Some workers consider that when insulin and posterior pituitary extract are administered simultaneously and subcutaneously the insulin does not exert its usual action since it is not absorbed owing to capillary constriction provoked by posterior pituitary extract. Such experiments seem to be beside the point, since the fact remains that in the presence of an already established hypoglycaemia posterior pituitary extract is effectual in restoring the blood sugar to normal. The writer and Mr. Nelson carried out a large series of experiments on cats under varying conditions, and came to the conclusion that the effect of posterior pituitary extract was mediated through the suprarenal glands, and that the central nervous system was not involved, the only prerequisite being an adequate store of liver glycogen. It is usually considered that the secretion of the suprarenal glands affords one means for the regulation of the blood sugar, but it would appear that posterior pituitary extract exerts an extra stimulus for the secretion of adrenalin. These results are to be published in *The Medical Journal of Australia*.

CLINICAL RELATIONSHIPS.

Thiourea and Thiouracil.

In the previous report mention was made of the laboratory's collaboration with the Honorary Medical Staff in the treatment of thyrotoxicosis by means of these two drugs. As indicated the results here have not been satisfactory, and in most cases toxic symptoms and relapses ensued. One patient was maintained in a satisfactory state for approximately two years, but at the end of that time severe peripheral neuritis occurred, and the drug had to be discontinued. Many of the Surgeons complain of the extreme vascularity of the thyroid when the above drugs are used as a preliminary to operation.

Sulphaemoglobinaemia.

During the year six patients suffering from Sulphaemoglobinaemia were investigated. In two instances large doses of an aspirin-phenacetin caffeine mixture had been administered. Previously to these cases only sporadic ones had been encountered, but the present series occurred within a few weeks of each other.

Growth Promoting Factor.

At the request of one of the Honoraries the growth promoting factor alleged to occur in heart muscle was prepared for clinical use. In the cases that were investigated it could not be stated with any certainty that beneficial results had occurred. In many cases rest in bed caused improvement, and it was difficult to evaluate any effect of the growth promoting factor.

The writer has again assisted in the classes of Clinical Biochemistry at the Melbourne University.

PUBLICATIONS.

A. B. CORKILL, DOROTHY J. POLLOCK and GRACE E. SMITH:

"The Value of Biochemical Tests in the Interpretation of Jaundice"—In Press (The Medical Journal of Australia).

A. F. DOUTCH and J. F. NELSON:

"The Effect of Morphine on Dye Concentration Curves for Plasma Volume Determination in Dogs"—In Press (The Australian Journal of Experimental Biology and Medical Science).

P. FANTL:

“The Bleeding Tendency in Obstructive Jaundice: Diagnosis and Management”—In Press (The Medical Journal of Australia).

P. FANTL and M. H. NANCE:

“An Acquired Haemorrhagic Disease in a Female due to an Inhibitor of Blood Coagulation”—In Press (The Medical Journal of Australia).

ROUTINE BIO-CHEMISTRY DEPARTMENT.

Again work in this department has been maintained at a steady level. The research staff has carried out many prothrombin estimations, and several interesting cases with abnormal androgen excretions were investigated.

The Mental Hospital at Mont Park has kindly supplied us with control specimens of cerebro-spinal fluid.

During the year the following tests have been carried out:

Blood Urea Estimations	459
Urinary Protein	229
Urea Concentration	231
Urea Clearance	221
Blood Sugar Estimations	412
Blood Sugar Curves	146
Benedict Tests	308
Acetone Bodies	305
Cerebro-spinal Fluid Examinations	407
Lange's Colloidal Gold Curves	30
Basal Metabolic Rate Determinations	180
Fouchet Tests	34
Van den Bergh Tests	48
Benzidine Tests	71
Pyramidone Tests	68
Urinary Diastase	29
Serum Calcium	6
Test Meals	467
Blood Chloride Estimations	3
Urine Examinations for Bilirubin, Urobilin, etc.	34
Prothrombin Estimations	9
Miscellaneous	173
	3,870
Electro-cardiographs	619
	4,489

The financial statement for the year is appended.

A. B. CORKILL, Director.

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE.
Balance Sheet At 31st December, 1945.

LIABILITIES.		ASSETS.	
Endowment Funds	£8,000 0 0	Cash at Commercial Bank of Australia Limited	£601 3 11
Capital—		Investments—	
Balance at 1st January, 1945	£1,092 0 9	Grain Elevator Board Inscribed Stock	£2,500 0 0
Add, surplus for year as per Revenue Account	1,609 3 2	Commonwealth Inscribed Stock, 3½% due 15/10/60	5,000 0 0
	2,701 3 11	Commonwelath Bonds, 3½% due 15/9/61	500 0 0
	£10,701 3 11	Furniture and Fittings	8,000 0 0
			2,100 0 0
			£10,701 3 11

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NOTE:—3½% Commonwealth Inscribed Stock, face value £17,000, is inscribed in the names of the Trustees of the Thomas Baker Estate for the benefit of the Institute.

We have audited the above Balance Sheet and certify it to be correct.

(Sgd.) FLACK AND FLACK,
Chartered Accountants (Australia),
Honorary Auditors.

Melbourne, 30th September, 1946.

Spectator Co., Printers, 134a Little Collins Street.
