

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

TWENTY-FIRST
ANNUAL
REPORT



1947

The Baker Institute derives its main financial support from the Thomas Baker (Kodak), Alice Baker, and Eleanor Shaw Benefactions. It is also dependent upon grants from The National Health and Medical Research Council, and donations from private sources. The latter are allocated to an Endowment Fund.

The Thomas Baker, Alice Baker, and Eleanor Shaw
Medical Research Institute

ALFRED HOSPITAL, PRAHRAN, MELBOURNE.

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- Mr. J. SUTHERLAND
-

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MRS. SHIRLEY E. SIMON, B.SC.

Routine Biochemical Department:

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MISS JEANNE M. UPFILL, B.SC.

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MISS NORMA BRIDESON.	MISS BEVERLEY HOARE.
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MISS JOAN FRANKS.	MISS JOAN POMROY.
MISS SUE GILLANDERS.	MISS JUDITH WILLIAMS.

Honorary Consulting Pathologist:

A. J. TRINCA, M.D., B.S. (MELB.), F.R.C.S. (ENG.), F.R.A.C.S.

Honorary Consulting Neurologist:

LEONARD B. COX, M.D., B.S. (MELB.), M.R.C.P. (EDIN.), F.R.A.C.P.

Honorary Electrocardiographist:

MORRIS C. DAVIS, M.D., B.S. (MELB.), F.R.A.C.P.

Honorary Assistant Electrocardiographist:

H. B. KAY, M.D., B.S. (MELB.), M.R.C.P. (LOND.), M.R.A.C.P.

The following Members of the Honorary Medical Staff of the Hospital are associated with investigational work:—

Thoracic Surgery:

C. J. OFFICER BROWN, M.D., B.S. (MELB.), F.R.C.S., F.R.A.C.S.

R. H. ORTON, M.B., B.S. (MELB.).

T. HAMLEY WILSON, M.S., F.R.A.C.S.

Cardiac Diseases:

H. B. KAY, M.D., B.S. (MELB.), M.R.C.P. (LOND.), M.R.A.C.P.

Gastric Diseases:

R. R. ANDREW, M.D., B.S. (MELB.), M.R.C.P. (LOND.), M.R.A.C.P.

Problems Pertaining to Carbohydrate Metabolism:

J. BORNSTEIN, M.D., B.S., M.R.A.C.P., Junior Research Fellow, The National Health and Medical Research Council.

The Director's Twenty-first Annual Report TO THE TRUSTEES

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

Gentlemen,

During this year the research work of the Institute has progressed along the line approved by you in 1946, namely, that problems of biochemical and physiological interest should obtain first priority. The reason for your decision was that the senior research staff had specialised experience in this type of work. The general policy has been to establish an efficient unit to carry out pure research with the proviso that if possible this should be developed in such a fashion that should occasion arise it could be diverted to the investigation of clinical problems. So far this scheme has proved eminently satisfactory both to the Hospital and the Institute.

Although the Institute cannot claim to have an attached clinical research unit, it must be pointed out that during the last few years there has been active co-operation with the Hospital in the investigation of clinical problems. Further, it is certain, as judged from requests that have recently been made by members of the Honorary Medical Staff, that the clinical liaisons of the Institute will be considerably extended. Some idea of what has been done, and what is contemplated, will be appreciated from consideration of the following.

As mentioned in my previous report, laboratory facilities were extended to Mr. C. J. Officer Brown and his Thoracic Surgery Unit for the purpose of animal experimentation to develop a technique for making systemic and pulmonary arterial shunts. It is of interest to note that some months ago, for the first time in Australia, two "blue babies" were successfully operated upon in the Alfred Hospital. In relation to this work, the Institute has carried out the blood gas analyses, and, in view of the fact that Dr. Kay, who joined the Institute as an associated worker, proposes to carry out cardiac catheterization, it is expected that estimations of the oxygen content of the blood will be in much greater demand.

Dr. R. Andrew has recently been given laboratory facilities for the investigation of gastric diseases. He is studying the influence of various drugs on gastric motility, and for this purpose a kymographic unit has been established.

The research work has gradually sorted itself out into two main sections. Dr. Fantl leads a team concerned with the investigation of the factors concerned with the clotting of blood and the study of blood diseases associated with either increased or decreased clotting tendencies. The intravascular clotting of blood either following surgical procedures or occurring spontaneously, presents an important clinical problem, and up to the present unanimity of opinion as to the causative factors is still lacking. In this connection the work of Link and his colleagues in America is of extreme interest. It was found that cattle in certain areas had abnormal bleeding tendencies. Sometimes after de-horning or castration the animals would bleed profusely. On investigation it was found that the animals were feeding on improperly cured clover, in which a substance now known as Dicoumarol had developed. This compound was shown to inhibit the synthesis of prothrombin, one of the essential factors concerned with the normal clotting of blood. It was therefore a natural step to synthesize Dicoumarol for the control of conditions due to abnormal clotting of the blood, and at the moment this compound is extensively used in America. Dicoumarol has, however, proved to be somewhat of a two-edged weapon of defence. It is a very potent drug, and the severe effects may be difficult to control.

Dr. Fantl was interested in this problem and prepared an ethylidene derivative known as 3,3'-ethylidene-bis-(4-hydroxycoumarin), (E.D.C.), which can be administered by mouth, and provided appropriate laboratory tests are available, a perfect control of blood coagulation can be attained. E.D.C. treatment has now become a routine procedure in this Hospital. I have purposely elaborated this subject in order to illustrate how pure research can be applied to clinical problems.

Apart from this work, another group of workers is engaged upon problems related to carbohydrate metabolism. The writer has always been interested in this aspect of research, and over the years a team has been developed to carry out such investigations. The practical application of the results to diabetes in man has always been kept in mind. Carbohydrate metabolism is closely related to that of fat, and Mr. Lincoln, under the supervision of Dr. Fantl, is concerned with problems dealing with

fat oxidation. The writer and Mr. Nelson have for some time been investigating various problems of carbohydrate metabolism; Dr. Bornstein is also concerned with the same problem, particularly in relation to diabetes. Under the appropriate sections this work will be reported in detail.

The Institute is again indebted to The National Health and Medical Research Council for financial support for the study of various problems. The Commonwealth Serum Laboratories has shown great co-operation in the supply of materials for the work of the Institute, and also Messrs. W. S. Kimpton and Sons have been very helpful.

Finally, I should like to thank the Will Trustees (Thomas Baker (Kodak), Alice Baker and Eleanor Shaw) for their continued generous support, which has served as a great stimulus to the scientific personnel of the Institute.

The Library.

Our thanks are due to the following for gifts of literature during the year: Bayer Products Ltd.; Commonwealth Serum Laboratories; The Connaught Laboratories; Council for Scientific and Industrial Research; Mr. Robert Fowler, F.R.C.S.; Graham Research Department, University of London; Imperial Chemical Industries of Australia and New Zealand Ltd.; Institut Bunge; Institute of Medical and Veterinary Science; International Anesthesia Research Society; The Editor, "Laboratory Journal of Australia"; The Mayo Clinic; Medical Research Council, London; Middlesex Hospital Medical School; Munitions Supply Laboratories; New York State Department of Health; Rockefeller Foundation; The Royal Australasian College of Surgeons; South African Institute for Medical Research; State Serum Institute, Denmark; Mr. A. J. Trinca, F.R.C.S.

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

PHYSIOLOGICAL AND BIOCHEMICAL RESEARCH

Studies on the Mechanisms of Blood Coagulation: Prothrombin Accelerator.

Dr. Fantl, Miss Nance and Mrs. Simon have continued their studies on prothrombin activation. For this work it is essential to have prothrombin preparations of a high degree of purity, and, as previously reported, barium carbonate and barium sulphate have the property of adsorbing prothrombin preferentially to other plasma proteins. By elution of the barium sulphate adsorbate with phosphate buffers of pH 7.8-8 it was found possible to obtain potent prothrombin solutions. With the use of isolated prothrombin it was shown that the speed with which it is converted to thrombin could not account for a normal blood coagulation time, and on continuing this line of investigation it was found that prothrombin-free plasma contained a factor which enhanced thrombin formation. This has been designated prothrombin accelerator. It was suggested from these observations that haemorrhagic tendencies connected with the prothrombin complex, could be due either to prothrombin deficiency or to a decreased amount of the prothrombin accelerator. This assumption has been verified by a report of Owren's concerning a patient with a haemorrhagic tendency. Owren designated the condition "parahaemophilia," and considered that it was due to lack of a hitherto unknown factor of blood coagulation which he termed Factor V. By means of various procedures, the details of which are described in an article entitled "The Physiological Activation of Prothrombin" (Med. J. A. 1 (5), 31st January, 1948, p. 128), Dr. Fantl and Miss Nance have isolated the prothrombin accelerator from the globulin fraction of oxalated plasma. Prothrombin- and fibrinogen-free preparations have been obtained, and it was shown that globulin has no effect on thrombin. Comparison of the properties of the accelerator with Owren's Factor V indicates that they are identical.

Several years ago Quick postulated from the results of experiments carried out on stored plasma that the prothrombin molecule consisted of two components, A and B, the former disappearing during storage. The properties of prothrombin A have not yet been defined, so that it is difficult to compare it with prothrombin accelerator. Recently Seegers and co-workers confirmed Dr. Fantl's findings and accepted his terminology. The activity of prothrombin accelerator in human plasma is less than that found in rabbit's plasma.

Recognition of the two factors involved in thrombin formation can be applied for the diagnosis of haemorrhagic conditions due to the prothrombin complex. Firstly, a prothrombin estimation is carried out in whole plasma and in plasma diluted with an equal volume of isotonic saline, using Quick's one-stage technique. If a delayed coagulation time is found the following possibilities have to be considered:

- (a) The bleeding tendency may be due to prothrombin deficiency, or
- (b) To normal prothrombin concentration, but a deficiency of prothrombin accelerator, or
- (c) Deficiency of both factors.

The answer will be found by carrying out prothrombin estimations in specimens obtained by adding to the patient's plasma an equal volume of normal plasma, from which prothrombin has been removed by adsorption on alumina gel or barium sulphate suspension:

- (a) If the prothrombin time in this test is similar to that obtained in the saline-dilution, then the diagnosis of hypoprothrombinaemia is established.
- (b) If, on the other hand, the plasma mixtures give shorter coagulation times than the saline-diluted plasma, a deficiency in prothrombin accelerator is present.

Intermediate results between (a) and (b) would indicate condition (c).

It should be pointed out that there is a discrepancy in the views of prothrombin activation. Whilst Owren maintains that prothrombin cannot be converted into thrombin in the absence of Factor V, we find that thrombin formation takes place without prothrombin accelerator, but the latter increases the speed of the thrombin formation. Further work is under way to clear up this point.

The Influence of Storage on the Coagulation Factors of Human Plasma.

It is known that whole blood or plasma loses its coagulability during storage even at low temperatures, which makes the use of stored plasma for transfusion purposes in haemorrhagic conditions of doubtful value. The procedures elaborated for the isolation of prothrombin and prothrombin accelerator have been applied to stored as well as fresh plasma in order to study the stability of these components during storage. It was possible to isolate prothrombin from plasma which had been stored for

four weeks, whilst the estimation of prothrombin activity in the plasma itself indicated that it was prothrombin-free. Further, it could be shown that prothrombin accelerator disappears gradually from stored plasma, the loss of activity being complete after four weeks' storage. The contention by some workers that an alteration of fibrinogen is responsible for the impaired coagulation time could not be substantiated. It is the loss of prothrombin accelerator together with an increase in anti-thrombic activity which is responsible for the delayed prothrombin time of aged plasma.

The Use of Fibrin Tubes for Repair of the Common Bile Duct:

So far the use of artificial tubes for the repair of the common bile duct has not been very satisfactory. Mr. J. Devine, F.R.C.S., has been carrying out animal experiments in which a new duct is produced from peritoneal grafts. This procedure requires some temporary support, and for this purpose fibrin tubes of various diameters and thicknesses were prepared. The idea underlying the application of these fibrin tubes is that they should give support for a sufficient period to establish the new duct and later they would be digested by proteolytic enzymes. The experiments carried out indicated that it was possible to preserve the fibrin tubes for periods up to three weeks.

The Use of Acid Phosphate in Cases of Chronic Osteoarthritis:

The suggestion referred to in the last annual report to use a mixture of M/15 primary and secondary sodium acid phosphates, with a pH 5.8, has been accepted by several physicians in this Hospital. Although beneficial results are reported following injections with this solution, estimations of calcium, inorganic phosphates, alkaline phosphatase, and citric acid in the blood serum of patients prior to and after the injection did not show any significant changes in these blood components.

Publications:

- P. FANTL, M. NOEL ROME and MARGARET H. NANCE:
"The Influence of Quinine Hydrochloride on the Plasma Coagulation Mechanism"—Aust. J. Exp. Biol. and Med. Sci., v. 25, 1947, p. 183.
- P. FANTL and M. NOEL ROME:
"Calcium and citric acid metabolism in bone diseases"—Proc. Roy. A/Asian. College Physicians, vol. 2 (2), July 1947, p. 52.

- P. FANTL and M. NOEL ROME:
 "The Relationship between Calcium and Citrate in Fowl's Blood"—Aust. J. Sci., v. 10 (1), August, 1947, p. 19.
- P. FANTL and MARGARET H. NANCE:
 "The Relation between Plasma Coagulation Time and Prothrombin Concentration"—Aust. J. Exp. Biol. and Med. Sci., v. 25, 1947, p. 95.
- P. FANTL and MARGARET H. NANCE:
 "The Hypoprothrombinaemic effect of 3,3'-ethylidene-bis-(4-hydroxycoumarin), E.D.C.: An Experimental and Clinical Study"—Med. J. A., v. 2, 2nd August, 1947, p. 133.
- P. FANTL and MARGARET H. NANCE:
 "The Physiological Activation of Prothrombin"—Med. J. Australia, v. 1 (5), 31st January, 1948, p. 128.
- P. FANTL and MARGARET H. NANCE:
 "Influence of Storage on the Coagulation Factors of Human Plasma"—In Press, Aust. J. Exp. Biol. and Med. Sci.

Carbohydrate and Fat Metabolism:

Dr. Bornstein has been carrying out investigations on the effect of high fat diets on rats rendered diabetic by alloxan. The observation of Burn, namely, that a high fat diet ameliorates alloxan diabetes, has been confirmed. In addition, it was found that in animals on a high fat diet, a renal factor was implicated. In these animals, despite a high blood sugar level, the urinary output markedly decreased when the high fat diet was commenced.

In addition, it was found that in contrast to alloxan diabetic animals not on a high fat diet and showing low liver glycogen values, those animals given a high fat diet showed no ketosis and although a high blood sugar was present, adequate liver glycogen reserves were present. This work is still in progress and a preliminary report will shortly be published.

Investigations on the insulin sensitivity of the alloxan diabetic rat were commenced with the view of elucidating the mechanism of hypoglycaemic reactions. However, rats proved unsuitable animals for this work, and it is proposed to use mice, in which the hypoglycaemic reactions are more clearly defined.

Another aspect of Dr. Bornstein's work is concerned with the detection of minute quantities of insulin in the blood stream. An original technique has been developed, and it is hoped that it will be possible to apply this to the measurement of blood insulin contained in man. Further, it is proposed to investigate

the influence of a high fat diet on the elimination of ketone bodies in diabetic patients receiving insulin.

Fat Metabolism:

The gravest metabolic disturbance in diabetes is the accumulation of the ketone bodies in the tissue fluids. This phenomenon is due to the excessive breakdown of fatty acids by the liver, and it therefore follows that the question of the chemical action of insulin in the mammalian organism may be elucidated by a more intensive attack on the problem of fat metabolism.

In continuation of the work referred to in the previous annual report the reaction products of palmitic, myristic and margaric acids have been identified.

Palmitate was dehydrogenated to the extent of 10-12% when judged by the iodine number of the reaction products. Ozonolysis and breakdown of the resultant ozonide yielded nonylaldehyde, indicating that $\Delta^{7:8}$ hexadecenoic acid has been formed. Similar treatment of margaric acid produced $\Delta^{8:9}$ heptadecenoic acid; in the case of myristic acid, the reaction product was $\Delta^{5:6}$ tetradecenoic acid. It is interesting to note that the double bond was introduced in each case between $C_9 - C_{10}$ when counted from the $-CH_3$ end of the chain. Palmitic and myristic acids yielded unsaturated fatty acids which are identical with the corresponding acids found in natural fats, suggesting that this mechanism plays some part in the maintenance of the ratio of saturated to unsaturated fatty acids in body fat.

Further experiments have been carried out, including aromatic and hydroaromatic compounds. In the case of beta phenyl propionic acid, dehydrogenation to cinnamic acid was observed, whilst cholic acid was not attacked.

Alloxan Diabetes:

The writer and Mr. Nelson have completed their investigations on alloxan diabetes, a preliminary note of which was included in last year's report. Further evidence confirming the views expressed at that time has been obtained.

While this work was in progress a report was noted in the literature which claimed that for alloxan to exert its diabetogenic effect it must first pass through the kidney, and while so doing a substance is produced which is responsible for the usual phenomena observed. Duff and co-workers repeated this experiment on rabbits, but could not confirm the findings of the earlier workers.

We were also investigating this problem when the results of Duff and his colleagues were published. Since our experiments on rabbits fully agreed with their work, we did not consider it worth while to proceed further.

It has also been shown that the main factor in the rapid breakdown of alloxan in blood is the low hydrogen ion concentration.

During the course of our earlier work, we noted that the diabetic condition of animals given alloxan subsequent to bilateral adrenalectomy did not appear to be as severe as that of unoperated animals.

To study this matter further, an investigation has been commenced on the blood sugar, urinary sugar, and ketone excretion before and after adrenalectomy of rabbits injected with alloxan.

Considerable difficulty has been experienced in producing a satisfactory preparation for this work since these animals are a very poor surgical risk. However, the results from the few successful cases show that the normal rabbit when given alloxan develops a high blood sugar and excretes large amounts of glucose and ketone bodies within a few days, resulting in rapid death unless insulin is given. These animals, subsequent to bilateral adrenalectomy, improve considerably in condition. The blood sugar falls to a level only slightly above the normal, urinary glucose slowly disappears, and the ketone body excretion falls to within normal limits. Further work to complete this study is now in progress.

Publications:

A. B. CORKILL:

“Carbohydrate Metabolism”—Proc. Roy Asian College Physicians, vol. 2 (2), July, 1947, p. 67.

A. B. CORKILL and J. F. NELSON:

“The Influence of Fructose on the Utilization of Glucose by Isolated Muscle”—Aust. J. Exp. Biol. and Med. Sci., v. 25, 1947, p. 347.

ANITA BROWN, A. B. CORKILL, P. FANTL and J. F. NELSON:

“Some Observations on Alloxan Diabetes in the Rabbit”—Med. J. A., v. 1 (7), 14th February, 1948, p. 197.

CLINICAL RELATIONSHIPS

During the year the Institute has again closely collaborated with the Honorary Medical Staff in the investigation of clinical problems. Some idea of the extent of collaboration, which it is hoped will develop further, will be realized from the introductory remarks concerning the work of the Thoracic Surgery Unit, and the investigation of gastric diseases.

In collaboration with Dr. Sewell, some interesting work was carried out on a patient suffering from hypoparathyroidism. Thyroidectomy was carried out on this patient twelve years ago, and she later developed typical parathyroid tetany. The usual lines of treatment, such as low phosphorus and high calcium diets, together with occasional injections of calcium gluconate, afforded some relief, but the patient's condition was very unstable. Through the Australian representatives of Bayer Pharma Pty. Ltd., supplies of "Dihydrotachysterol" were made available, and, following the oral administration of this compound, the patient has been free from tetany for the last eight months. The principle underlying the administration of Dihydrotachysterol is that it increases the absorption of calcium via the intestine. During its administration careful controls have to be made both on the serum calcium and the urinary calcium output.

As previously reported, laboratory facilities were granted to the Honorary Pathologist to the Institute, Mr. A. J. Trinca, and he is still carrying out research on the histology of breast cancer on behalf of the Central Cancer Registry.

For over six months the Institute, in association with the Neurological Clinic, has been concerned with the treatment of the intractable nervous disease, disseminated sclerosis. Based on the report by Putnam of success in preventing exacerbation of the disease by reducing the blood prothrombin level with Dicoumarol, about 30 patients have been treated with E.D.C. as recently synthesized by Dr. Fantl.

In a few cases there has been difficulty in satisfactorily reducing the blood prothrombin level, but in the majority this has been possible. In a group of patients in which the disease had been, before treatment, steadily progressive, this progression continued, despite the attainment of low blood prothrombin levels. In the group in which the course of the disease was marked by recurrent exacerbation, this continued to occur in a few cases despite low blood prothrombin levels. In the majority, no further exacerbation was reported, but the time is as yet too short to claim that this is the result of treatment. The investigation therefore will be continued.

ROUTINE BIOCHEMISTRY DEPARTMENT

The work of this department has again been maintained at its usual steady level. There has been some fluctuation in the number and variety of tests carried out. Sulphonamide estimations have not been in any demand whatsoever, but prothrombin estimations have greatly increased. This is due firstly to the fact that 3,3'-ethyldene-vis-(4-hydroxycoumarin), E.D.C., is now being extensively used for the treatment of thrombotic states, and, secondly, that cases of disseminated sclerosis have been treated under the supervision of Dr. Cox.

The demand for serum phosphatase determinations and liver function tests has also steadily increased.

We are again indebted to Mont Park Mental Hospital for the supply of control specimens of cerebro-spinal fluid.

During the year the following tests have been carried out:

Androgen Estimations	17
Basal Metabolic Rate Determinations	317
Blood Chlorides	36
Blood Cholesterol	30
Blood Sugar Curves	188
Blood Sugar Estimations (with Benedict Tests and acetone bodies estimations)	93
Blood Sugar Estimations (single)	620
Blood Sulphonamide Estimations	31
Blood Urea Estimations	343
Blood Uric Acid	22
Calculus Examinations	11
Cerebrospinal Fluid Examinations:	
For Total Protein	344
For Globulin	237
For Chlorides	257
For Sugar	29
Lange's Colloidal Gold Curves	60
Fouchet Tests	27
Van den Bergh Tests	152
Benzidine Tests	42
Fat in Faeces	11
Pyramidone Tests	1
Gastric Contents	10
Hanger's Cephalin Flocculation Tests	82
Hippuric Acid Excretion Tests	51
Prothrombin Estimations	1,281

Urea Clearance Tests (Blood urea, urinary protein, urea concentration)	419
Urea Concentration	4
Serum acid phosphatase	67
Serum Alkaline Phosphatase	71
Serum Calcium	36
Serum Protein Estimations of:	
Total Protein, Albumen and Globulin	256
Test Meals	418
Urine Examinations	136
Urinary Diastase	24
Vital Capacity	5
Electrocardiographs	945
Miscellaneous	139
	<hr/>
	6,812
	<hr/>

Financial statements for the year are appended.

A. B. CORKILL, Director.

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

Balance Sheet at 31st December, 1947.

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LIABILITIES				ASSETS			
Commercial Bank of Australia Ltd.	£605	7	6	Sundry Debtors	£329	8	10
Endowment Funds	8,000	0	0	Investments—			
Capital Grants and Gifts	700	0	0	Grain Elevator Board In-			
Capital—				scribed Stock	£2,500	0	0
Balance at 1/1/1947	£2,649	12	3	Australian Commonwealth			
Less Deficiency for Year				Inscribed Stock, 3½%,			
as per Revenue Account	1,525	10	11	due 15/10/60	5,000	0	0
	<hr/>			Australian Commonwealth			
		1,124	1 4	Treasury Bonds, 3½%,			
				due 15/9/61	500	0	0
					<hr/>	8,000	0 0
				Furniture and Fittings	2,100	0	0
		<hr/>				<hr/>	
		£10,429	8 10			£10,429	8 10

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 Institution.

