

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

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FIFTEENTH  
ANNUAL  
REPORT

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1940-41



## PREFACE.

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The year 1941 has been an eventful one for the Institute. For some time there has been great difficulty in finding room for our ever expanding work. When acquainted with this shortage of laboratory space, the Trustees of the Will of the late Mr. Thomas Baker generously laid aside sufficient money to enable another story to be put on the building, and, in spite of the adverse times and conditions, we hope to have it soon under way, and our routine and research work much enhanced by such an extension.

This year has also seen the formation of an Advisory Council to the Trustees. Both they and the Director are greatly indebted already to it. The names of the Advisory Council will indicate how fortunate the Institute is in having such help.

The Institute mourns the death of its first Director, Dr. Penfold. Since his retirement from directorship of the Institute in 1938, he remained on the staff as Consultant Bacteriologist. Dr. Penfold came to the Institute with a world-wide reputation for research in bacteriology and with the very great experience gained as Director of the Commonwealth Serum Laboratories he bent his whole energies to shaping the Baker Institute on the best lines of research, and has left an imperishable name on its records. A short memorial notice by his colleague and friend, Dr. Willis, will be found in the body of the Report.

J. F. MACKEDDIE,  
Chairman of the Trustees.

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

**ALFRED HOSPITAL, PRAHRAN, MELBOURNE.**

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**THE TRUSTEES OF THE INSTITUTE.**

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- Dr. BALCOMBE QUICK .. Member of the Board of Man-  
agement of the Hospital.
- J. SUTHERLAND, Esq. ... Director of Kodak (A/sia) Pty.  
Ltd.
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\*Prof. W. T. YOUNG.

Sir DAVID RIVETT.

Dr. IVAN MAXWELL.

Dr. JOHN KENNEDY.

Assoc. Prof. W. DAVIES.

Prof. MACALLUM.

\*Since going to press, Professor Young died after a short illness.  
His death is a severe blow to the Institute. His ever willing  
help was of great assistance to the Director.

Honorary Treasurer :

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Honorary Solicitor :

JOHN TURNBULL, Esq. (Blake & Riggall).

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Lieut.-Col. J. H. P. ELLER, D.S.O., V.D.

Director :

Dr. A. BASIL CORKILL.

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**Full-time Workers under the National Health and Medical  
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A. H. ENNOR, M.SC., Physiological Research.

CHARLOTTE M. ANDERSON, M.SC. (Resigned), Physiological  
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M. NOEL ROME, B.SC., Physiological Research.

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ROSE WYSOKIER, B.SC., Biochemist.

**Consulting Bacteriologist:**

W. J. PENFOLD, M.B., CH.M. (EDIN.), D.P.H., B.HYG. (DUNELM),  
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ALFRED J. TRINCA, M.D., B.S. (MELB.), F.R.C.S. (ENG.), F.R.A.C.S.

**Honorary Electrocardiographist:**

DR. M. C. DAVIS, M.D., B.S.

# The Director's Fifteenth Annual Report

TO THE TRUSTEES

of the

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

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Gentlemen,—

The present report covers the activities of the Institute from 1st May, 1940, to 31st December, 1941. This departure from the usual twelve months period was necessitated by your decision to bring the scientific year of the Institute into line with the financial year. Future reports will, therefore, cover the twelve months commencing 1st January.

Since the last report there have been many changes in the staff. Following the decision, referred to in my last report, to transfer the routine bacteriology to the new Pathology department, I have to inform you that this was effected on the 1st July, 1940. This work is now in Dr. Willis's department, and is under the control of Miss Jean Tolhurst, M.Sc. Miss Tolhurst joined the Baker Institute in 1935, and for a considerable period collaborated with Dr. Penfold in preparing a stable toxoid for active immunisation against gas gangrene organisms and tetanus. In addition, she participated in the work of the routine bacteriological department, thus gaining a very wide experience. We shall greatly miss the services of so excellent a worker.

In January of this year Miss Petherick, B.Sc., joined the bacteriological staff of the Institute. In collaboration with Dr. Singer, she is investigating the factors concerned in natural resistance. With the consent of Professor Woodruff, under whose general supervision the work is being carried out, the results are to be submitted as a thesis for the M.Sc. degree. I greatly appreciate the facilities that have been granted from time to time by the University authorities for carrying out extra-mural work in his Institute.

In October of this year the scientific world suffered a great loss by the death of the first director of this Institute, Dr. W. J. Penfold. Incorporated in this report is a biographical note on Dr. Penfold by Dr. Willis, and I feel sure that you all will endorse the opinions therein expressed. The sound policy laid down by Dr. Penfold in the early days of the Institute have proved of inestimable value in the development of the research work. Personally, I owe him a debt of gratitude for my early training in scientific research.

Another great loss to the Institute occurred when Mrs. Haab, for private reasons, found it necessary to relinquish her post as Secretary to the Director. Mrs. Haab was associated with the Institute from the start, and devoted fifteen years to loyal and efficient service. Her knowledge of languages, coupled with her skill as a typist and stenographer, rendered her a most valuable member of the staff.

In June of 1940 Mr. Wilkins, for reasons of health, found it necessary to resign from his position as Trustee of the Institute, and his place was taken by Dr. Balcombe Quick. Mr. Wilkins's services will be greatly missed.

On 1st May, 1940, at the instigation of the Chairman, Dr. Mackeddie, a most important step was taken by the formation of an advisory committee to the Director and Trustees. The original members of this committee were Professor Osborne, Dr. G. Morgan (Director of the Commonwealth Serum Laboratories), Professor W. Young and Associate Professor Davies. At a later date Sir David Rivett, Drs. John Kennedy, Ivan Maxwell, and Professor Macallum accepted invitations to join the committee.

Apart from establishing valuable relationships with the scientific institutes which the various members represent, it is stimulating to have access, for the purpose of discussing problems, to men of such varied scientific interest. In particular I must express my thanks to Dr. Morgan and Associate Professor Davies. Dr. Morgan has, at all times, most willingly supplied materials for bacteriological research, and Associate Professor Davies has assisted Dr. Singer by the preparation of various organic compounds for chemo-therapeutic studies.

In March of this year Miss Charlotte Anderson, M.Sc., resigned from the staff in order to attend the medical course at the University of Melbourne. Miss Anderson first joined the Institute as a voluntary worker in 1936. The results of her investigations on the relation of the anterior pituitary gland to carbohydrate metabolism were accepted as a thesis for the M.Sc.

degree. Following this, Miss Anderson received research grants successively from the University of Melbourne and the National Health and Medical Research Council. Her work was of a very high standard. Miss Noel Rome has been appointed to take Miss Anderson's place.

We are again indebted to the National Health and Medical Research Council for research grants. This body has continued to support the work of Mr. Ennor and Miss Rome.

#### **The Library.**

We gratefully acknowledge gifts of literature during the year from the following:—Mr. Robert Fowler, F.R.C.S.; The Mayo Clinic, Rochester, N.Y.; The Rockefeller Foundation; New York Academy of Medicine; Middlesex Hospital Medical School; South African Institute for Medical Research, Johannesburg; Henry Lester Institute, Shanghai; The Medical Research Council, London; The Commonwealth Health Department; The Science Museum Library, London; The Walter and Eliza Hall Institute; Turkish Central Hygiene Institute, Ankara, Turkey; Connaught Laboratories, University of Toronto, Canada; The Department of Physiology, University of Queensland; New York State Department of Health; Public Health Department, New South Wales; Public Health Department, Victoria; The Institute of Medical and Veterinary Science, South Australia.

### **WILLIAM JAMES PENFOLD.\***

#### **A Biographical Note by Dr. R. A. Willis.**

The recent death of Dr. W. J. Penfold terminated the career of one of Australia's greatest medical scientists.

Born on 27th September, 1875, at Brampton, in Cumberland, Penfold passed his final examinations in medicine with honours at Edinburgh before his twentieth birthday. As he could not graduate until he was 21 years of age, he proceeded to the Continent for a year's study. He visited Berlin and Vienna, and in the latter city he studied pathology and bacteriology under Albrecht and Ghon respectively. He became rapidly proficient in German, in which language he always remained fluent, even to the extent of addressing medical meetings and taking part in discussions.

Returning to Edinburgh to graduate in 1896, he began practice by spending three years as a medical officer in mental

\*This is an abridged version of an article appearing in the "Medical Journal of Australia," 6th December, 1941, reproduced through the courtesy of the editor of that journal.

hospitals, where he made over 200 post-mortem examinations of the brain. Subsequently he had a large and varied experience in clinical practice in Newcastle-on-Tyne. His first paper, on mitral and tricuspid incompetence, was published during this period. From the first Penfold felt strong leanings towards the scientific and laboratory rather than the clinical aspects of medicine; and in 1909, in spite of heavy domestic responsibilities, he went to the Lister Institute, London, first as a voluntary research worker, later as a British Medical Association scholar. In 1912 he was appointed a member of the bacteriological staff of the Institute, then under the direction of Charles Martin. During his stay at the Institute he also held a number of other London appointments, namely, Pathologist to the Victoria Hospital for Children, where he personally conducted over 500 post-mortem examinations; Pathologist to the Dental Hospital, Leicester Square, and Lecturer on Bacteriology in the Dental School of this hospital. He was also a lecturer in the University of London, and was for some time Assistant Editor on the staff of the Bulletin of Tropical Diseases Bureau.

At the Lister Institute Penfold soon proved himself to be a scientific bacteriologist of the first rank, his papers on bacterial variation (1910-12), on the mechanism of fever (in collaboration with Hort, 1911-13), and on the lag phase of bacterial growth (partly in collaboration with Ledingham, 1914) gaining him world-wide recognition. In 1915 he and Ledingham, who later succeeded Martin as Director of the Lister Institute, were deputed to undertake the pathological services of the King George Military Hospital at Waterloo, the medical division of which was in the charge of Dr. C. V. Mackay, now of Melbourne. This hospital, containing nearly 2000 beds, and receiving many Australians invalided to England with dysentery and other infections acquired in Gallipoli and Mesopotamia, gave Penfold a wide practical experience. During this period he also visited Salisbury to investigate the epidemic of cerebro-spinal fever amongst the Canadian troops, and to carry out immunisation work. He was also largely responsible for designing the first mobile bacteriological laboratory used in the war.

Penfold's acceptance in 1916 of the invitation of the Commonwealth Department of Health to become the first Director of the Commonwealth Serum Laboratories at Royal Park was an event doubly fortunate for Australia, not only providing this country with a supply of vaccines, sera and other biological products of a quality equal to any in the world, but also giving us one of the best scientific brains we have had. The establishment and early development of the Serum Laboratories called

for a rare combination of great scientific knowledge and good administrative ability. Penfold fortunately possessed this, and the growth of the laboratories under his direction was rapid and successful. It would be difficult to enumerate all the practical details of manufacture and technique which he developed in the work of the laboratories, but special mention must be made of his introduction of the beneficial practice of returning the blood corpuscles to horses after bleeding them for sera. The eleven years of his directorship were years of arduous practical and administrative work, giving him little scope for pure research; but even so he found time for papers on such diverse subjects as the chemical properties of the pneumococcus, the types of tubercle bacillus prevalent in Australia, and adjustable microscope tube, and the nature of cancer.

While his work at the Commonwealth Laboratories was necessarily of a practical nature, Penfold did not lose the research spirit. Hence it was not surprising that, when in 1926 he was invited to become first director of the new-born Baker Research Institute at the Alfred Hospital, he accepted this post. There now began for Penfold a period of intense activity in medical research of a very varied kind, mainly in collaboration with various members of his staff and of the clinical staff of the Hospital. These researches were not only in bacteriology, but also in physiological chemistry, physics, veterinary medicine and parasitology, with a direct bearing on some specific medical problem. They were tackled with the same scientific precision and thoroughness as the more abstruse and academic problems of his Lister Institute days. Every branch of the laboratory and clinical work of the Hospital felt the stimulus of his thought and scientific outlook. I personally, as pathologist to the Hospital, soon found how profitable and stimulating it was to discuss my problems with him, for his outlook was of that broad kind which transcends the details of any one subject and makes its possessor an invaluable mentor in all fields allied to his own.

Of the many subjects which interested him during his directorship of the Baker Institute, several deserve special comment. His first task was to greatly improve the efficiency of the routine laboratory bacteriology and biochemistry of the Alfred Hospital. While Penfold himself speedily ensured this for the bacteriological section, a similar service in the biochemical section was effected by the work of Dr. A. B. Corkill, who was the senior member of his institute staff, and who later succeeded him as Director.

Active immunisation against anaerobic bacillary infections was a problem constantly in his mind. In 1930, in a paper with Parker on black disease in sheep, he envisaged the possibility of the prevention of gas gangrene in man, and from 1935 onwards he collaborated with Miss Jean Tolhurst in a sustained research which completely demonstrated that man as well as laboratory animals could be effectively immunised against *Bacillus Welchii* by two injections of an alum-precipitated formol-toxoid. He was disappointed that the military authorities did not see fit to give this method an extensive practical trial amongst the troops in the present war.

In 1934, on behalf of the Melbourne and Metropolitan Board of Works, Penfold undertook an extensive research on the subject of taenia saginata infestation in the population of Victoria and the possible risk of infection from the consumption of beef grown at the Werribee sewage farm. In collaboration with his son, Dr. H. B. Penfold, and with Miss Mary Phillips, this research was planned and executed with characteristic thoroughness. It not only proved the alleged risk of the consumption of beef raised on the Werribee farm to be negligible, but also led to some significant facts regarding the life history of the parasite and the development of natural immunity in animals infected by it. It must be added here that, unfortunately, as is often the case, the political action which was taken was not based on the scientific findings.

Penfold's retirement in 1938 was necessitated by his failing health, dating from a severe and almost fatal attack of hemiplegia over two years earlier. In spite of this and of several subsequent attacks, he retained his mental alertness and scientific interest almost to the last. He attended meetings of the Victorian Pathological Society up to a few months ago, and one of his last conscious acts was to write a letter on eclampsia, which appeared in the "Medical Journal" for 25th October, on the day preceding his death.

Of Penfold's personal qualities the most outstanding was his uncompromising love of truth. Meticulous scientific accuracy distinguished his own work and thought, and he was outspoken in condemnation of what he deemed its lack in others. He hated diplomacy, subterfuge and compromise; and, having once made a decision which he believed right on any issue, he was inflexible. This attitude was found to offend many and to make positive enemies of some, for, being human, his own judgment sometimes erred. To those who really knew him, however, his scientific earnestness and probity could never be doubted; and to those

of whose sincerity he was sure he gave generously of his store of scientific knowledge and his friendship. Penfold's love of scientific truth largely constituted his personal philosophy, and rendered social success and religious orthodoxy alike needless. His fearlessness was apparent in his candour, even when he knew that his opinions would be unwelcome and likely to be to his own material disadvantage.

He was a versatile reader, especially of history and general classical literature. He loved Shakespeare; and he read many modern works also with keen enjoyment and a remarkably retentive memory. While music was denied him because of marked tone deafness, he appreciated art of the classical and realist styles, and when possible encouraged sincere young artists.

He frequently spoke to his friends of his wife's loyal support and encouragement to him in his work; and, while his fortitude during his last four years of repeated illnesses was characteristic of him, it clearly owed much to the same source.

## PHYSIOLOGICAL AND BIOCHEMICAL SECTION.

### Staff:

Dr. A. B. Corkill.  
Dr. P. Fantl.

Mr. A. H. Ennor.  
Miss Noel Rome.

### Carbohydrate Metabolism.

#### Glucose Production by the Liver.

The investigations reported previously have been continued and extended. The validity of the assumption that liver phosphatase is not completely inhibited by high concentrations of inorganic phosphate has been carefully tested. Liver pulps, containing negligible amounts of glycogen, were incubated with hexose-monophosphates in the presence of M/45  $\text{PO}_4$  buffer pH 7.2, and M/4  $\text{PO}_4$  buffer pH 7.2.

In both series of experiments the breakdown of the added hexose-monophosphates was the same, indicating that the liver phosphatases were not greatly inhibited by a high concentration of inorganic phosphate.

However, the breakdown of glycerophosphate added to similar liver preparations was completely inhibited by M/4 or M/6 inorganic phosphate buffer.

This illustrates the differential behaviour of liver phosphatases towards different phosphoric acid esters. From the

work of Folley and Kay, and King and Delory, it is apparent that the more acid the organic phosphate the more readily it is hydrolysed. Therefore, the dissociation constant of the substituted phosphoric acid would be a decisive factor in determining the affinity between the ester and the enzyme. Glucose-1-PO<sub>4</sub> and hexose-6-PO<sub>4</sub> are stronger acids than glycerophosphoric acid, and, therefore, show a greater affinity for the phosphatase system, and the link between the hexose-monophosphates and the enzymes cannot be severed by inorganic phosphate.

Pure samples of glucose-1-PO<sub>4</sub> and hexose-6-PO<sub>4</sub> required for these experiments were prepared by Kiessling's method. Extracts of rabbit muscle, poisoned with iodoacetic acid, were incubated with starch instead of glycogen. After 10-15 minutes' incubation, glucose-1-PO<sub>4</sub> could be obtained, and after a longer incubation period (45 mins.) the Barium salt of hexose-6-PO<sub>4</sub> could be isolated. These salts were further purified by alkaline and acid hydrolysis respectively.

The steps involved in the formation of blood sugar from glycogen by the liver include two or, possibly, three successive enzymatic reactions.

Glycogen + PO<sub>4</sub><sup>'''</sup>  $\rightleftharpoons$  glucose-1-PO<sub>4</sub> ( $\rightleftharpoons$  glucose-6-PO<sub>4</sub>)  $\rightleftharpoons$  Glucose + PO<sub>4</sub><sup>'''</sup>. Previous studies concerning these reactions were carried out under conditions remote from the physiological in order to identify the intermediates involved. Dr. Fantl and Miss Rome have determined the conditions for this enzyme complex "in vitro" under conditions approximating those in the normal intact liver cells. The enzyme responsible for the breakdown of glycogen is present mainly in the lyoform. From the minced livers of both fasting and well-fed rabbits 70-100% of the enzyme system can be extracted by shaking with phosphate solution or water.

If glycogen and M/45 PO<sub>4</sub> buffer be added to such extracts the sole end product is glucose.

The optimum pH for this reaction was found to be at pH 6.6 + 0.1, declining on both sides of the apex. This curve differs from the pH activity curve obtained for muscle extracts by Cori et al., and Bauer et al, independently. Muscle extracts, containing phosphorylase and phosphoglucomutase, show a broad maximum activity between pH 7 and 8, whereas the liver system shows a much diminished activity in this range.

However, the curve of the reaction velocity of purified muscle phosphorylase obtained by Cori is very similar to that found for liver extracts.

That slight changes of pH have such marked influence on activity of this system suggests that it may have physiological significance, and could explain why, in acidosis, the blood sugar is higher than normal. Support for this view is given in observations of Guest and Rawson, who found that liver glycogen tends to vary directly and the blood sugar inversely with the increase in alkalinity of the blood up to pH 7.3.

The enzyme systems concerned with glycogen breakdown in liver and muscle differ in that muscle contains no phosphatase, whereas liver phosphatase is strongly reactive.

In order to see how the breakdown of hexose-monophosphates would influence the pH activity curve, extracts from glycogen-free livers were incubated with hexose phosphates in the range pH 5.6-7.6. Whereas the breakdown of glucose-1-PO<sub>4</sub> increased markedly with decreasing acidity of the reaction mixture, the hydrolysis of hexose-6-PO<sub>4</sub> was independent of the pH in the investigated range. The breakdown of glucose-1-PO<sub>4</sub> is accompanied by the conversion of this ester into the hexose-6-isomer by phosphoglucomutase.

Lehmann observed that the addition of free glucose to muscle extract inhibits the enzyme phosphorylating glycogen and Soskin stressed the importance of this fact for the liver system. He suggested that glucose is a homeostatic factor controlling the level of blood sugar.

All enzymes are inhibited, to some extent, by the reaction products, which means that the reaction constant would fall off during the incubation time. The falling off of the reaction constant may be due either to partial destruction of the enzyme at the reaction temperature or to the inhibiting influence of glucose. Lowering the temperature did not appreciably alter the reaction rate, and, under favourable experimental conditions, it was possible to show that the reaction involving the conversion of glycogen to glucose obeys the laws of a reaction of the first order. This implies that glucose, except in high concentrations and remote from the physiological, cannot inhibit glycogen breakdown, and, therefore, Soskin's theory of a homeostatic mechanism for glucose formation in the liver cannot be accepted.

### **Vitamin K.**

In response to a request from clinicians of the Alfred Hospital for supplies of a compound with vitamin K activity, Dr. Fantl prepared 2-Methylnaphthoquinone, a substance which is known to have the same potency as the natural vitamin K. As this compound is fat soluble, it appeared likely that it would

be absorbed through the skin. Accordingly, an oily solution was prepared and administered in the form of an inunction.

The first case in which this procedure was adopted was one of obstructive jaundice in which operation had been performed. Two days later slight but persistent bleeding from the incision developed. The plasma clotting time, estimated by the method of Quick, was forty seconds, as compared with twenty seconds in the case of two controls. This, from Quick's data, corresponds to a prothrombin content of about 20%. An area of skin of approximately 100 square centimetres was cleaned with ether, and one cubic centimetre of a 1% solution of 2-methyl-1, 4-naphthoquinone in cod-liver oil was rubbed in with a glass spatula. Absorption appeared complete after three hours. Twenty-two hours later the plasma clotting time had fallen to 22 seconds and all bleeding had stopped. In another case of obstructive jaundice the prothrombin content of the plasma estimated pre-operatively was 37% of the normal (clotting time 27 seconds), and 22 hours after inunction it had risen to 80% (clotting time 20.5 seconds). In a case of a premature child with neonatal haemorrhage, 0.5 millilitre of the solution was applied on two successive days. On the third day all clinical symptoms of jaundice had disappeared, but, unfortunately, no prothrombin determinations were carried out.

Up to the present seven additional cases of obstructive jaundice before and after operation have been observed. In six, normal plasma clotting times were found, and therefore no necessity for application of vitamin K arose. In the seventh case the clotting time was 22 seconds before inunction, and 20.5 seconds 27 hours after inunction.

The inunction has been applied to four normal persons, and in no case was there any change in the plasma clotting time, nor could any untoward symptoms be observed.

Since not many patients with vitamin K deficiency came to our notice, it was decided to study the value of the percutaneous vitamin K application on animals deficient in this vitamin. These experiments, undertaken by the writer and Dr. Fantl, however, were discontinued because of a coinciding publication of de Beer and co-workers who have shown that 2-methyl-1, 4-naphthoquinone dissolved in various organic solvents is absorbed by the skin of chicks which had previously been maintained on a diet deficient in vitamin K. The prolonged clotting time of the blood of these birds was considerably reduced by this treatment. Thus it can be assumed safely that the rise

in prothrombin level in the cases mentioned was due to absorption, through the skin, of the quinone.

In view of the suggestion made by several workers to administer preparations with vitamin K activity to all women in the last days of gestation in order to prevent neonatal haemorrhage, the inunction should be considered as a simple, effective and cheap procedure.

### **Sex Hormones and Sterols.**

The natural plant sterols, Cholesterol and Stigmasterol, are important starting materials for the synthesis of Progesterone and other hormones. W. Bergmann found, several years ago, that American bivalves contain an isomer of Stigmasterol called Ostreasterol. In view of these findings, it was thought worth while to investigate Australian mollusca in order to see whether they would be a source of such compounds. Dr. Fantl undertook the investigations of the non-saponifiable matter of the common mussel.

Mussels collected in Port Melbourne occasionally contained up to 25% Cholesterol. However, the Cholesterol content is subject to variation since specimens collected at other times were free of it. The Provitamin D content was found to be, on the average, 5% of the non-saponifiable matter. The highest figures for Provitamin D were obtained by an alkaline hydrolysis and subsequent extraction with benzene. Much lower values were found by acetone-ether extraction because the long extraction period necessary for the removal of the lipid matter destroyed the sensitive Provitamin D. The presence of Provitamin D was determined colorimetrically by the Rosenheim reaction, and corroborated by measurements of the ultra-violet absorption spectrum. We were greatly indebted to the Director of the Commonwealth Serum Laboratories, Dr. Morgan, for providing facilities for these measurements, and we are very grateful to Drs. W. Hurst and Traill for the photographic measurements and their evaluation. A few other invertebrates were also investigated. Compared with other foodstuffs, the bivalves investigated were found to be the richest source of Provitamin D. Apart from Cholesterol and Provitamin D, two other sterols were isolated as tetrabrom acetates, the properties of which are different from the hitherto described sterols. Neither Stigmasterol nor Ostreasterol could be detected. The estimation of the molecular weight of the purified sterol-acetates was carried out by the saponification method of Sandqvist and Bengtson. Frequently, however, only small amounts of material were available. In order to carry out accurate estimations, a weight burette

was designed which, operated by compressed air, gave satisfactory results.

### **The Influence of the Anterior Pituitary Gland on Metabolism.**

In the last Annual Report some mention was made of the work being carried out by Mr. Ennor and Miss Anderson on the influence of anterior pituitary extracts on the oxidation and reduction of glutathione. This work has been completed, and it was found that glutathione was present in the oxidised form in the livers of rabbits suffering from a toxæmia and also in normal and adrenalectomised animals undergoing treatment with anterior pituitary extracts. Such extracts, although having a decided effect on the rate of oxidation of glutathione, had no demonstrable effect upon reduction rates.

Further work has been done on the diabetogenic action of the anterior pituitary gland. Reference has already been made to the difficulties encountered in the production of a permanent diabetes in dogs. Since the extracts used were active, in that they contained a fraction whose action was similar to that described by Young, of Hampstead, no satisfactory explanation of the failures was forthcoming, although it was realised that the potency of the extracts was probably rather low. In a report to the National Health and Medical Research Council in 1939, the occurrence, in these animals, of an anti-diabetogenic hormone was suggested. Such an anti-hormone, if present, would explain the failures previously mentioned. With this suggestion in mind, Dr. Singer collaborated with Mr. Ennor so that the serological aspects of the problem could be investigated. Serum was collected from rabbits which were subjected to bi-weekly injections of a partially purified saline extract of anterior pituitary gland. Complement fixation with the serum was carried out in the usual manner by testing serial dilutions of the serum with serial dilutions of the antigen. Serum diluted 1/1700 gave definite zonal fixation with crude saline extracts of the anterior pituitary gland diluted between 1/80 and 1/320. Partially depancreatized rats were used as the test animals, and, after a preliminary control period, during which time urine was collected for the estimation of glucose, intraperitoneal injections of anterior pituitary extract were commenced. This resulted in a marked increase in the urinary glucose, which was maintained over the course of the experiments. On the other hand, animals which received serum in addition to the anterior pituitary extract showed no such behaviour. The glucose excretion, in place of increasing, fell to zero levels, a behaviour which showed that not only did the serum protect against the diabetogenic

action of the extract, but also against the glycosuria resulting from loss of the major portion of the pancreas. These results have been confirmed and extended in order to gain a more intimate knowledge of the mechanisms involved. In this connection, prolactin (pH 4.5 precipitable fraction) growth and thyrotrophic factors were prepared from fresh glands and used for the absorption of the serum. The experiments described above were repeated, and it was found that there was complete protection against the diabetogenic action of the anterior pituitary extract in those animals treated with unabsorbed serum, some protection in the case of the serum absorbed with the growth and thyrotrophic factors, but absolutely no protection when serum absorbed with prolactin was administered. The slight loss in protective action of the serum following absorption with growth and thyrotrophic factors is almost certainly due to the partial removal of protective antibodies by these relatively impure preparations. It is considered that traces of prolactin may constitute the impurity, although it is possible that further contaminants, not identical with the known fractions of the anterior pituitary gland, were present. The complete inactivation of the protective serum following absorption with prolactin denotes removal of antibodies contained in the serum, and thus establishes the fact that the diabetogenic and prolactin factors are similar as regards their antigenicity, and, in addition, that there is probably no serological relationship between the diabetogenic factor and the growth and thyrotrophic principles.

The question, whether these hormonal fractions have a species specificity, has also received attention by these two workers. Antibodies against ox serum protein were removed from the protective serum, which was then divided into two portions. These were then absorbed with ox and sheep prolactin respectively, and tested for protective action in partially depancreatized rats. The loss in protective power of the serum absorbed with sheep prolactin was found to be as great as that of the serum absorbed with an equivalent amount of ox prolactin.

In experiments with depancreatized rats protected against the diabetogenic action of anterior pituitary extract by sera partially absorbed with the pituitary fractions, it was found that absorption with the pH 4.5 precipitable fraction resulted in the removal of all protective antibodies whilst absorption with the other fraction containing the thyrotrophic and gonadotrophic hormones did not interfere with the protective action of the serum. In agreement with this result, it was found that the rise in blood sugar in the dog following the injection of crude

anterior pituitary extract could be produced by the injection of the pH 4.5 precipitable fraction. Injection of the pH 4.5 non-precipitable fraction, on the other hand, was without effect.

Definite proof of the presence and action of an antidiabetogenic hormone would, of course, be obtained if it were possible to produce the following two experimental results:—

1. Demonstration of the antibodies against the anterior pituitary extract in the serum of a dog at the time at which the animal recovers from the diabetic state in spite of continued injections.

2. Demonstration of the power of serum from such an animal to protect another animal against the diabetogenic action of anterior pituitary extract.

At the present time, although the results are few in number, the first phenomenon has been shown to occur.

The serum of such dogs is capable of protecting depancreatised rats against the diabetogenic effect of anterior pituitary extract and shows a moderate content of complement fixing antibodies against anterior pituitary extract in "in vitro" experiments. A single intraperitoneal injection of the equivalent of 5 gm. of fresh anterior pituitary gland will produce a definite rise in the blood sugar of the dog within four hours, whilst extract kept at room temperature for twelve hours is without effect. The hyperglycaemic effect of the anterior pituitary extract is completely neutralised if the extract is mixed with the antiserum and kept at 0° C. for 24 hours prior to injection. Only one experiment was performed in which the serum was injected 24 hours prior to the anterior pituitary extract, but no protective action could be demonstrated. This negative result is, in all probability, due to the small dose (50 ml.) of serum administered.

During the treatment necessary for the production of the serum from the dogs an interesting phenomenon was observed. All the animals under investigation exhibited an extreme sensitivity towards the hyperglycaemic effect of the anterior pituitary extract after 4-6 daily injections. This sensitivity was so marked that in some experiments the blood sugar level was raised quite appreciably after the injection of as little as the equivalent of 0.5 gm. of fresh gland. Such animals have proved excellent test objects for the diabetogenic action of partially purified anterior pituitary extract, and it is hoped that they

will aid materially in the attempts at the isolation of the diabetogenic factor in a purer state than has hitherto been possible. As far as this aspect of the work is concerned, it is interesting to note that it has been possible to illustrate the hyperglycaemic effect of the corticotrophic fraction of the anterior pituitary gland. Here the injection of 30 mg. of corticotrophic hormone (equivalent to 50 gm. fresh gland) was followed by a 35 mg. increase in blood sugar level. This result should not be taken as indicative of the belief that the corticotrophic fact is identical with that producing the hyperglycaemic effects, but rather that these two factors are linked in some manner with the phenomenon of diabetes.

### **Fat Metabolism.**

In addition to the investigations just described, Mr. Ennor has carried out studies on the livers of guinea-pigs which have been injected with extracts of the anterior pituitary gland, which are particularly potent in producing fatty livers. A definite accumulation of fat in the liver can be observed less than three hours following injection of the equivalent of 1.5 gm. of fresh gland. Together with this increase in hepatic lipid, there is also an increase in ketone body production, but, curiously enough, no increase in the oxygen uptake of the liver tissue. In fact, even when the fat content reaches 20%, the maximum value so far obtained, there is only an insignificant increase in the oxygen consumption. This result is in direct contrast to those obtained (see later) with the livers of animals treated with carbon tetrachloride and phosphorus.

Mr. Ennor has confirmed Best's work in the rabbit and guinea-pig, where it was shown that the fat accumulating in the liver after the injection of anterior pituitary extract disappears in five days or so, in spite of continued injection of the extract. This effect is being thoroughly investigated, and, whilst some light has been thrown upon the problem, the results are not in a sufficiently advanced stage to permit of discussion.

Following a single injection of anterior pituitary extract, the fat content in the liver increases to reach a maximum in 18-30 hours, after which it slowly decreases to normal in 100 hours. This latter figure represents the maximum time for complete recovery; the minimum has not yet been determined.

Another interesting phenomenon has been encountered here, and that is that the ketone body production lags behind the disappearance of fat from the liver. For instance, it is found that, although the fat content has fallen to normal values, and

remained there for at least 24 hours, the ketone body production is still 3-4 times greater than that found in untreated livers.

The response of the livers from animals treated with anterior pituitary extract to added fatty acid is strictly comparable to that seen in the normal, carbon tetrachloride, and phosphorus fatty livers.

### **Metabolism of Fatty Livers Produced by Phosphorus and Carbon Tetrachloride.**

The following observations were made:—

1. There is an increased oxygen uptake in the liver slices in spite of marked hepatic damage. This increased oxygen consumption appears to be correlated with fat content, and generally is only seen when the latter exceeds 9-10% (normal 5-7%). Moreover, the fact that other workers in the field have been unable to find this increased oxygen consumption can be explained by the method of calculation of results. Generally the oxygen consumption is referred to unit dry weight of the tissue, and, whilst there is no objection to so expressing the results in the livers of normal animals where the fat content shows little variation, it is evident that erroneous conclusions may be drawn when the fat content increases to 3-5 times the normal value.

2. There is no diminished ability on the part of the fatty liver to oxidise added fatty acids. This finding completely negatives the suggestion made by some workers that the increase in fat, under the influence of carbon tetrachloride and phosphorus, is due to the derangement of dehydrogenation mechanisms.

3. Before the increase in consumption of oxygen is apparent there is a very marked (2-10 fold) increase in hepatic ketogenesis, which takes place as soon as the fat content increases over the normal range.

4. Determination of the iodine numbers of the liver fat has confirmed the finding (2) that there is no derangement in dehydrogenation mechanisms. If such were the case there would be a greater amount of saturated fat in the liver than normally; however, no difference could be found.

5. Valeric acid (C<sub>5</sub>) behaves differently to the even carbon acids. The latter acids, when added to liver slices, give rise to ketone bodies, but valeric acid fails to do so, and in addition completely inhibits their formation in a liver which is normally producing large amounts.

## Insulin Inactivation by Sulphydryl Compounds.

Mr. Ennor and the writer have completed these investigations, and it can now be stated that the action of cystein in preventing the blood sugar decrease following insulin injections is not related to any action that this sulphydryl compound may have on insulin "in vitro." The conclusion was one which was suggested in our publication, "Effect of Sulphydryl Compounds on the Action of Insulin 'in Vivo,'" "Australian Journal of Medical Science," volume 18, page 379, 1940.

It has further been established that cystein has a marked action in increasing the blood sugar—an increase which masks the blood sugar lowering action of insulin. This effect is produced through an action of cystein on the adrenal glands, as have been proven by the use of adrenalectomised rabbits. As further proof, it was found that in animals treated with ergo-toxine, no hyperglycaemic effect of cystein was apparent.

### Publications.

A. B. CORKILL:

"Adrenal Cortex," "Medical Journal of Australia," 20/9/41, p. 324.

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

"The Influence of Adrenal Cortical and Sex Hormones on Liver Glycogen," "Australian Journal of Experimental Biology and Medical Science," 19, 241, 1941.

"The Influence of Fructose upon the Peripheral Utilisation of Glucose," "Australian Journal of Experimental Biology and Medical Science," 18, 171, 1940.

A. B. CORKILL and A. H. ENNOR:

"The Influence of Sulphydryl Compounds on the Action of Insulin," "Australian Journal of Experimental Biology and Medical Science," 18, 379, 1940.

A. B. CORKILL, A. H. ENNOR and J. F. NELSON:

"Insulin Inactivation by Sulphydryl Compounds," "Australian Journal of Experimental Biology and Medical Science" (in the press).

A. H. ENNOR:

"The Influence of Anterior Pituitary Extract in the Detoxication Mechanisms of the Dog," "Australian Journal of Experimental Biology and Medical Science," 18, 163, 1940.

"On the Oxidation of Fatty Acids in the Liver," "Australian Journal of Science," 4, 27, 1941.

A. H. ENNOR and E. SINGER:

"Serological Protection Against the Diabetogenic Hormone of the Anterior Pituitary Gland," "Journal of Physiology" (in the press).

A. H. ENNOR and C. M. ANDERSON:

"The Influence of Anterior Pituitary Extracts upon the Rate of Oxidation and Reduction of Glutathione in Tissues," "Australian Journal of Experimental Biology and Medical Science," 19, 69, 1941.

P. FANTL:

"The Estimation of Sulphanilamides and Other Primary Aromatic Amines in Body Fluids," "Australian Journal of Experimental Biology and Medical Science," 18, 175, 1940.

"A Simple Weight Burette for Use in Organic Analysis," "Australian Journal of Experimental Biology and Medical Science," 19, 279, 1941.

"The Sterol Fraction of Australian Marine Mollusca," "Australian Journal of Experimental Biology and Medical Science" (in the press).

P. FANTL and A. B. CORKILL:

"Percutaneous Treatment of Vitamin K Deficiency," "The Medical Journal of Australia," 8/11/41, 1941, p. 540.

P. FANTL and C. M. ANDERSON:

"The Breakdown of Hexose Monophosphates in Liver Pulp," "Australian Journal of Experimental Biology and Medical Science," 19, 117, 1941.

P. FANTL, C. M. ANDERSON and J. F. NELSON:

"Concerning Glycogen Breakdown in the Liver," "Australian Journal of Experimental Biology and Medical Science," 18, 369, 1940.

## BACTERIOLOGICAL SECTION.

Staff:

Dr. E. Singer.

Miss M. Petherick.

### **Virus Cultivation in Cell Free Media.**

In a previous paper ("Australian Journal of Experimental Biology and Medical Science," 19, 123, 1941) experiments were reported on the survival of Rickettsiae in cell free media. Further attempts were made to separate the substances which support virus growth from organ extracts. As the method of Lee, Hisaw and Cohn was applied with success to the separation of the different antigenic hormones of the pituitary, it was used for this study. The working hypothesis directing this study was that the substances supporting virus growth are probably very labile and easily destroyed by tissue enzymes. Quick and sharp separation, therefore, seemed essential.

Accordingly, separation was effected in the cold by the slow addition of ammonium-sulphate through a rotating cellophone membrane.

Different types of extracts and fractions were tested for their growth-supporting action for influenza virus. The virus strains used were either directly isolated from mouse lung or passaged in tissue culture. For these experiments a strain of influenza virus was maintained for over 60 generations in two different types of tissue culture. Maitland's method of embryonic chick-Tyrode medium and Zinssers medium were employed.

Whilst the results of these experiments are definitely encouraging in that it has been possible to maintain a low titer virus for 3-5 generations in cell-free media, no prolonged propagation of virus could be obtained.

### **Serological Studies on the Hormonal Antigens of the Anterior Pituitary Gland.**

To some extent these were carried out in collaboration with Mr. Ennor, and are reported in the biochemical section. It appears that the hormones of the anterior pituitary gland are the only ones against which antibodies of true protective action can be produced. Antibody formation of a specific character can be obtained against a number of other biologically active substances of hormonal or enzymatic nature, i.e., insulin, thyroglobulin or pepsin. These antibodies, however, whilst giving specific precipitin reactions or complement fixations with their homologous antigen, are devoid of protective action.

Furthermore, even the pituitary hormones can be prepared in a non-antigenic state.

According to Marrack's theory, an antigen containing only one determinant group would be inferior, as regards its antigenicity, to one containing more; consequently it should be easier to protect against substances of the latter type. It seemed likely, therefore, that the structure of the pituitary hormones could be visualised as follows. A fairly large protein molecule on which are distributed several smaller groupings responsible for the biological activity. On the other hand, it could be imagined that only one active grouping is present in those substances against which serological protection cannot be obtained.

To test the possibility of such a mechanism, the behaviour of artificial antigens was studied. Different compounds containing strongly reactive groups on an inert nucleus were prepared. The nuclei consisted of phenol, naphthol, resorcinol and protein respectively. The determinant group was p-amino phenyl arsenic acid radicle linked to the nucleus by diazotisation and coupling. The phenol and naphthol compounds contained one arsenic group, the resorcinol two and three, and the arsenic protein several. In accordance with the working hypothesis, serological protection could be obtained against the resorcinol and protein antigens, but not against those containing only one arsenic radicle.

It thus appears that the difference between the pituitary hormones and others, such as insulin, adrenalin, etc., in so far as their antigenicity is concerned, could be adequately explained by the difference in the number of determinant grouping carried by the respective antigens.

#### **Chemo-Therapeutic Studies.**

Three valuable chemo-therapeutic compounds have the general constitution  $X \langle \text{---} \rangle \text{NH}_2$ , where X signifies an acid radicle. The substances, namely, sulphanilamide, atoxyl and stibamine, differ only in respect of the chemical element which constitutes this radicle, sulphur, arsenic and antimony in the respective compounds.

The chemo-therapeutic action of these three compounds differs according to the nature of the acid radicle. Sulphanilamide is active mainly against bacteria, atoxyl and stibamine mainly against protozoa.

It was thought that the results obtained by testing compounds analogous to the three described, but differing in the

nature of the acid radicle, would constitute a valuable addition to knowledge of the chemo-therapeutic action of this type of compound.

Several selenium compounds prepared by Dr. Davies were, therefore, tested against streptococcal and influenza virus infection in mice. These compounds had the following chemical constitution:—

1.  $\langle \rangle \text{SeO}_3\text{NH}_4$
2.  $\text{NO}_2 \langle \rangle \text{SeO}_3\text{NH}_4$
3.  $\text{NH}_2 \langle \rangle \text{SeO}_3\text{NH}_4$
4.  $\text{CH}_3\text{CONH} \langle \rangle \text{SeO}_3\text{NH}_4$

In addition to the ammonium salts from compounds 2-4, the potassium salts were also tested.

Compound 1 was found inactive, compound 2 was highly toxic, and compounds 2 and 4 had a very slight action on streptococci and a doubtful one on influenza virus infection in mice.

Recently compounds belonging to the group of the selenones were prepared by Dr. Davies, and are now being tested. This type of compounds—analogueous to the sulphones—should be more active than the compounds previously tested.

#### Studies on Natural Resistance.

These investigations are being carried out by Miss Petherick, who intends to submit her results as a thesis for the M.Sc. degree. Her findings may be summarised as follows:—

Rats and mice exhibit a resistance to C.diphtheria and its toxin of such a high order as to be very nearly absolute. In an attempt to elucidate the mechanism of the resistance of the mouse, work has been carried out to compare the action of diphtheria toxin on the naturally resistant mouse and on the susceptible guinea-pig.

In tests carried out "in vitro" to determine the relative amounts of diphtheria toxin destroyed by organ pulp (liver-spleen) of guinea-pigs and mice, guinea-pig organs did not destroy toxin within 24 hours when it was diluted with saline, glucose saline (0.1, 0.3 and 0.6% glucose), and only after 48 hours in tyrode-serum (nine parts of tyrode to one part serum). Mouse organs destroyed toxin diluted with tyrode, serum tyrode and glucose saline (0.1%) in 24 hours. The process concerned in the destruction of diphtheria toxin by mouse organs was not

activated by glutathione. Guinea-pig leucocytes suspended in saline and in serum-tyrode destroyed more toxin than rat leucocytes suspended in saline and serum-tyrode. Hence the natural resistance cannot be due to the leucocytes, but must be located in the organs.

Three and five hours after intradermal injection of toxin, slightly more toxin could be extracted from the skin of guinea-pigs than of mice.

The fatal dose of toxin for mice was the same whether the toxin was injected intravenously or intraperitoneally. The ratio between the intracerebral fatal dose and the intraperitoneal fatal dose was 1 : 7 for both guinea-pig and mouse.

Urinary excretion of toxin continues right up to death in the case of guinea-pigs, whilst mice excrete toxin only during the first six hours following injection.

When toxin was injected intravenously it was found to remain in the serum of mice and guinea-pigs after it had disappeared from the organs. Less toxin was recovered from the organs and serum of guinea-pigs than of mice.

It is, therefore, probable that the resistance of mice is not caused by one single factor only, but rather by a multiplicity of mechanisms.

The main factor seems to be that the tissues of mice destroy toxin more quickly than the organs of guinea-pigs.

## **Publications.**

### **E. SINGER:**

"A Note on the Treatment of Gas Gangrene with Sulphanilamide and Related Compounds," "Medical Journal of Australia," 8/6/40, p. 796.

"Experimental Studies on the Combined Sulphanilamide and Serum Treatment of Gas Gangrene Infections," "Medical Journal of Australia," 28/9/40, p. 275.

"A Note on the Influence of Surgical Operations on the Diphtheria Antitoxin Content of Blood Serum," "Medical Journal of Australia," 17/5/41, p. 613.

"Experiments on the Survival of Rickettsiae in Cell Free Media," "Australian Journal of Experimental Biology and Medical Science," 19, 123, 1941.

"Studies on the Hormonal Antigens of the Anterior Pituitary Gland," "Australian Journal of Experimental Biology and Medical Science," 19, 125, 1941.

"Studies on Blood Preservation," "Medical Journal of Australia," 25/5/40, p. 724.

W. J. PENFOLD, J. C. TOLHURST and D. WILSON:

"Active Immunisation Against Gas Gangrene and Tetanus," "Journal of Pathology and Bacteriology," 52, 187, 1941.

### ROUTINE BIOCHEMISTRY.

Miss J. Marks.

Miss R. Wysokier.

Mr. Max Swann (on Active Service).

The amount of work has retained its high level, though we have tried to control it as much as possible owing to the difficulties in obtaining chemicals and apparatus.

The number of curves done for the Diabetic Clinic has been reduced, a fasting sugar being estimated and a curve carried out only if the single sugar is within normal limits. About 140 blood sugar curves have been done on recruits for the Royal Australian Air Force. Most of these were lag curves or low threshold in type. A few of the men showed mild diabetic curves on first examination, but normal curves on repeating the test. When glycosuria was detected in the medical examination many of these men were found to have been worried and nervous. Of the total number, about five gave true diabetic curves.

There has been a marked increase in the number of the renal function tests—urea concentration, blood urea and urea clearance tests. The urea clearance test has been found to be a much better index of the kidney function than the older tests.

Since the opening of the neuro-surgical ward the number of cerebro-spinal fluids received for chemical examination has increased considerably.

During the past year a member of the Royal Australian Air Force has been in the laboratory to learn the simpler biochemical technique, also we have had a senior sick bay attendant from the Royal Australian Navy.

Dr. Fantl, of the research staff, has carried out a considerable number of prothrombin investigations on cases of suspected

Vitamin K deficiency. In addition, this worker has estimated urinary androgen excretion in patients exhibiting the basophilism syndrome of Cushing. These estimations were required to rule out the presence of an adrenal cortical tumour.

During the period, May, 1940, to December, 1941, the following tests have been carried out for the Hospital:—

	May to Dec., 1940	Jan. to Dec., 1941	Total
Fractional Test Meals . . . . .	359	567	926
Blood Urea Estimations . . . . .	372	561	933
Urinary Protein Estimations . . . . .	96	311	407
Urea Concentration Estimations . . . . .	167	338	505
Urea Clearance Tests . . . . .	132	302	434
Blood Sugar Estimations . . . . .	166	350	516
Blood Sugar Curves . . . . .	166	174	340
Blood Sugar Curves for R.A.A.F. . . . .	42	93	135
Benedict Tests . . . . .	496	416	912
Benedict Tests for R.A.A.F. . . . .	126	212	338
Acetone Tests . . . . .	549	432	981
Acetone Tests for R.A.A.F. . . . .	126	217	343
Cerebro-spinal Fluid Examinations . . . . .	221	390	611
Lange Colloidal Gold Curves . . . . .	120	216	336
Basal Metabolic Rate Estimations . . . . .	83	107	190
Basal Metabolic Rate Estimations for R.A.A.F. . . . .	—	6	6
Fouchet Tests . . . . .	35	52	87
Van den Bergh Tests . . . . .	40	51	91
Benzidine Tests . . . . .	85	149	234
Pyramidon Tests . . . . .	23	133	156
Diastase Tests . . . . .	20	36	56
Blood Calcium Estimations . . . . .	19	14	33
Blood Cholesterol Estimations . . . . .	10	9	19
Vital Capacity Estimations . . . . .	10	2	12
Alkali Reserve Estimations . . . . .	11	5	16
Miscellaneous . . . . .	106	84	190
	<hr/>	<hr/>	<hr/>
	3,580	5,227	8,807
Electrocardiographs . . . . .	473	737	1,210
	<hr/>	<hr/>	<hr/>
	4,053	5,964	10,017

The financial statement for the year is appended.

A. B. CORKILL,

Director.

THE THOMAS BAKER, ALICE BAKER AND ELEANOR SHAW MEDICAL RESEARCH

Financial Statement 1st January to 31st December, 1941.

RECEIPTS.

Balance, 1st January, 1941 ..	£324	19	11
Thomas Baker (Kodak), Alice Baker			
Eleanor Shaw Benefactions ..	5784	9	2
Department of Health ..	493	10	0
Donations—Dr. Boan ..	3	3	0
Donations—Dr. Boan ..			
1st (£746/10/7)—			
Australian Consolidated			
Urban ..	£637	10	0
London Elevator Board ..	93	15	0
London Hospital ..	15	5	7
Proceeds of Sale of—			
Paravatus ..	5	2	0
Photographs ..	22	15	1
Lithographs ..	77	8	0
Annual Fees ..	215	6	2
	<u>7348</u>	<u>4</u>	<u>0</u>
Balance, 31st December ..	7673	3	11
	1455	5	9
	<u>£9128</u>	<u>9</u>	<u>8</u>
Amount held—Grain Elevator Board			
Balance ..	£2500	0	0

By Balance at 31st December, 1941

EXPENDITURE.

By Medical Salaries ..	£2,992	1	1
Other Salaries and Wages ..	4,291	1	1
Drugs, etc. ..	251	1	1
Instruments, Glassware, etc. ..	403	1	1
Special Maintenance ..	633		
Fuel and Lighting ..	129	1	1
Insurance ..	23	1	1
Repairs ..	52	1	1
Library ..	184	1	1
Printing, Stationery, and Postages ..	30		
Travelling ..	6	1	1
Sundries ..	127		

I have audited the above Statement and certify it to be correct.

(Signed) FLACK & FLACK,

Chartered Accountants, 23/5/42.

