

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

SIXTEENTH
ANNUAL
REPORT

1941-42

PREFACE.

Last year was a very broken one. One of our research workers, Mr. Ennor, was released for Chemical Defence service. Our Director himself was loaned for months at a time for similar duties. The routine work for the Hospital was maintained intact, and as much research work was taken on as the curtailed staff could manage.

Our Board of Trustees has been increased from three to five—the two additional members being also members of the Trustees of the Will that control the finance of the Institute—Mr. J. C. Gates, representing the Trustees of the Will, and Mr. Edgar Rouse, the Hospital Board. With this closer contact of the Settlers with the work of the Institute and the expected completion of the additional story to the building, the coming year should be one of marked progress.

In another place reference is made to our great loss in the death of Professor Young, an outstanding member of our Advisory Committee.

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Chairman of the Trustees.

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Medical Research Institute**

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A. H. ENNOR, M.S.C., Physiological Research (at present working
for the Chemical Warfare Section of the Munitions Supply
Laboratories).

M. NOEL ROME, B.S.C., Physiological Research.

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JEAN P. MARKS, PH.C., DIP. BIOCHEM. ANALYSIS (LONDON),
Biochemist.

ROSE WYSOKIER, B.S.C., Biochemist.

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

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

The Director's Sixteenth Annual Report

TO THE TRUSTEES

of the

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

Gentlemen,—

In common with other research institutes, our scientific work has been profoundly modified by war requirements. A considerable amount of research has been done on behalf of the Chemical Defence Board of the Munitions Supply Laboratories, and for obvious reasons the results of these investigations cannot at present be recorded.

In particular, physiological research has been disorganised owing to Mr. A. H. Ennor and the writer being released to attend a special course of instruction on chemical warfare physiology. In June of this year Major Gorril, R.A.M.C., instituted this work at the Melbourne University, and the writer has been more or less continuously engaged on various problems associated with Chemical Warfare. Further, Mr. Ennor has been given leave of absence for the duration of the war to work for the Chemical Defence Board of the Munitions Supply Laboratories.

Miss Mary Petherick, who, as mentioned in my previous report, has been carrying out research work on problems of natural resistance, obtained her Master of Science degree when her results were submitted as a thesis.

This year Miss Noel Rome, B.Sc., in collaboration with Dr. Fantl and the writer, is investigating the role of renal ischaemia in the production of experimental hypertension. The work is being carried out under the general supervision of Professor Wright, who has agreed for Miss Rome to submit her results as a thesis for the degree of Master of Science.

The Institute greatly misses the services of the late Professor Young, a member of the Advisory Committee. Professor Young

was one of a brilliant line that laid the foundation of distinguished research work in the Lister Institute. To him and Harden is due the modern development of carbohydrate metabolism. His first Australian contact with research was when appointed Biochemist to the Australian Institute of Tropical Medicine at Townsville, where he studied the metabolism of the white race in the tropics. Seven years later we find him lecturer in biochemistry at the Melbourne University, to become its first Professor in 1908. Here, to his other great gifts, he added that of a distinguished teacher, and is held in grateful memory by several generations of students. Besides his professional duties, he undertook vital research work in association with the Council of Scientific and Industrial Research, and amid those ever-increasing calls on his time and strength he found it possible to come on the Advisory Committee of the Baker Research Institute. Here his ripe wisdom in all matters of research was of inestimable value. And all this with a modesty and quiet demeanour that made association with him a unique and delightful experience.

We are again indebted to the National Health and Medical Research Council, who have supported the work of Miss Rome and Miss Petherick.

Finally, I must express my thanks to Mr. Douteh for his ever willing and original technical assistance given to all members of the staff of the Institute. Without his help in the construction of apparatus, etc., various problems could not have been carried out.

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, Melbourne; New York State Department of Health; The Institute of Medical and Veterinary Science, South Australia; The Royal Melbourne Hospital.

PHYSIOLOGICAL AND BIOCHEMICAL SECTION

Staff:

Dr. A. B. Corkill.

Miss Noel M. Rome.

Dr. P. Fantl.

Mr. J. F. Nelson.

Mr. A. H. Ennor.

Vitamin K.

The role played by this substance in the formation of prothrombin is now well recognised. Approximately two years ago Dr. Fantl prepared 2-methylnaphthoquinone, which acts in a similar manner to the natural Vitamin K, and continuous supplies have been available to the medical staff of the Hospital. Excellent results have been obtained by administering 2-methylnaphthoquinone to patients in whom bleeding occurs owing to a deficiency of blood prothrombin. The compound, according to our suggestion, is put up in oily solution and administered by injection. Details of our results are published elsewhere.

Coumarin Compounds.

"Sweet clover" disease has been extensively investigated by a group of workers in America. Cattle fed on spoilt or improperly cured clover develop hypo-prothrombinaemia, and consequently a tendency to haemorrhages, which often end fatally. K. P. Link has shown that the causative agent in spoilt clover is 3,3' Methylene bis (4 hydroxy coumarin). The substance has been synthesized, and is known as Dicumarol. The mode of action of Dicumarol is not known. "In vitro" it does not inhibit the coagulation of blood. Theoretically, Dicumarol could be broken down to salicylic acid, and Link considers that this might explain its mode of action.

In support of this theory, he states that if salicylic acid is administered to rats kept on a Vitamin K deficient diet, hypo-prothrombinaemia develops. It is well known that substrate competition occurs with compounds having certain essential groups in common, i.e., p-amino-benzoic acid and sulphanilamide. With the idea that a similar mechanism may be concerned in the action of Dicumarol, Dr. Fantl synthesized a number of compounds having some structural relationship to either Vitamin K or Dicumarol. The following substances were prepared, i.e..

- (1) 3 methyl—4 hydroxycoumarin.
- (2) Methylene bis (dihydro di-methylresorcinol).
- (3) 3,3' ethylidene, bis (4 hydroxycoumarin).

These compounds, in slightly alkaline solution, were injected intraperitoneally into rabbits maintained on a normal diet. Derivative No. (1), given in doses 200 times greater than the amount of Dicumarol required to produce hypo-prothrombinaemia, was inactive. This, therefore, invalidates the idea of substrate competition to explain the action of the Dicumarol. No. (3) compound was the only one active in producing hypo-prothrombinaemia, but it is much less potent than Dicumarol. Not only are larger amounts required to produce a demonstrable effect, but the duration of the hypo-prothrombinaemia is about 24 hours as compared with, say, 3-4 days in the case of Dicumarol. Theoretically this is of advantage since it suggests a safer method of maintaining hypo-prothrombinaemia than with Dicumarol. If this latter agent is used, as advocated in America, to replace Heparin, there is always the risk of the therapy getting out of control, with ensuing haemorrhages.

Carbohydrate Metabolism.

In the last report it was stated that the enzyme system present in the liver, which is responsible for the production of glucose from glycogen, has a pH optimum at about 6.6. Most of these experiments were carried out on livers containing blood. In further work, Dr. Fantl and Miss Rome have studied the influence of pH on glucose production in extracts of perfused rabbit livers. In some experiments a definite divergence from the pH activity curve was observed. It was found that the addition of 0.7×10^{-3} M Mg, a concentration approximately that found in the normal liver, restored the system to its optimal activity.

The element Mg is known to be essential for several enzymatic reactions in carbohydrate metabolism. We assume that it activates the first step in the degradation of glycogen to glucose-1-phosphate. On the other hand, zinc or manganese ions in a concentration 10^{-3} M in liver extracts, in the presence of low concentrations of inorganic phosphate, always diminished the glucose production. However, since there is no accumulation of the hexose-6-phosphate in the reaction mixtures, it is concluded that these ions inhibit the activity of the liver phosphorylase.

These results need a more detailed investigation, since they are in contrast to findings with muscle phosphorylase. Here zinc and manganese do not interfere with the action of the enzyme. It has to be pointed out, however, that most of the experiments with muscle extracts were carried out in higher phosphate concentrations. It remains to be seen whether there

is a real difference of the two phosphorylases, or whether higher concentrations of phosphate have a protective influence on them.

It is known that noble metals inhibit phosphorylase activity, and it is also possible that zinc and manganese, in the presence of low concentrations of phosphate, inhibit the liver phosphorylase by blocking essential groups in the enzyme molecule.

Glucose-1- and hexose-6-phosphate are important substances for studies in carbohydrate breakdown. They have been prepared according to the technique previously reported by incubating starch with fresh muscle extracts poisoned with iodoacetic acid. With most commercial starches no difficulties have been encountered in obtaining good yields of glucose-1-phosphate, but with certain samples of wheat starch we failed to isolate hexose-6-phosphate in the expected amounts. An analytical study indicated that all starches showed a total phosphorus uptake not differing by more than 10 per cent. Thus it is evident that the first step in the degradation yielding glucose-1-phosphate was not inhibited.

However, the ratio of 1 to 6 ester was significantly changed in the case of "wheat starch," which suggested that this starch either contained an inhibitor of phosphoglucomutase or lacked an accelerator which was present in the other starches.

It was found that the ash of the "wheat starch" exerted an inhibitory influence on the conversion, whereas the "potato ash," added to the wheat starch, had no accelerating influence on the formation of hexose-1-phosphate.

Spectrographic analyses of the ashes of the starches, for which we are grateful to Mr. Keith Winsor, showed that, whereas all the starches contained traces of Ca, Cu, Mg, Mn, P, Si and B, only the wheat starch contained Zn, suggesting that the zinc content of the wheat starch was responsible for the inhibition of the phosphoglucomutase. This experience can be usefully applied in the preparation of hexose phosphates.

The addition of 2×10^{-5} M zinc is of advantage in the preparation of glucose-1-phosphate, whereas in the case of the preparation of hexose-6-phosphate, the addition of 2×10^{-3} M manganese is advisable, to abolish any inhibition by zinc impurities.

On the request of the Medical Equipment Control Committee and the Chemical Defence Board of the Munitions Supply Laboratories, the Institute has carried out several investigations.

For obvious reasons, no details can be given. Dr. Fantl studied several steps in the synthesis of local anaesthetics of the p-amino benzoic acid groups with the view to finding economical processes with available raw materials. The experiments have been successfully concluded, and the processes handed over to the authorities, so that in case of emergency these essential drugs could be manufactured in this country.

Experimental Hypertension.

An enormous amount of research has been carried out by American and South American workers on the above subject. Following the production of renal ischaemia, the events leading to hypertension are postulated as follows:—

Renin liberated by kidney cortex reacts with blood globulins to form hypertensin—the vasoconstrictor principle. Although it is by no means certain, there is some clinical evidence to suggest that in some forms of hypertension in man similar factors to those in experimental hypertension may be present.

Miss Rome has carried out a series of investigations designed to produce a method for obtaining uniformly potent renin preparations. Her results may be summarised as follow:—

The use of organic solvents, such as acetone and alcohol, is inadvisable in the preparation of vasopressor renal extracts, since the extracts obtained under similar experimental conditions varied in potency from nil to 50,000 cat units. Furthermore, these solutions sometimes contained depressor factors. Saline extracts of renal cortex always contained the vasopressor principle, although frequently the pressor effect was preceded by a fall in blood pressure. It was found, however, that most of the depressor factors were precipitated from the saline solutions by acidification to approximately pH 4, whereas the amount of vasopressor principle in the solutions was unchanged.

The results obtained show that this latter technique yields renin preparations which have a purity and potency at least equal to, if not greater than, those of other workers. Further, this acid-saline method has several important practical advantages:—

- (a) Its reliability to give purely pressor preparations.
- (b) An extract is ready for testing within 24 hours after the kidney has been removed from the animal.

- (c) There is no difficulty in keeping the preparation cold during the whole time, and hence the labile active principle is retained.
- (d) The use of denaturing organic solvents is avoided.
- (e) The amount of impurity as judged by the nitrogen content of the solution is relatively small.

Influence of Anterior Pituitary Gland on Metabolism.

Work on the influence of the extracts from the anterior lobe on carbohydrate have been continued, although they have not been completed because of the transference of Mr. Ennor from the Institute to the Chemical Warfare Board of the Munitions Supply Laboratories, for the duration of the war.

In the last report mention was made of two experimental results which, if obtained, would largely constitute proof of the presence and action of the antidiabetogenic hormone. Firstly, it has been stated that if the results of the previous work in as much as the inability of the extracts to produce a permanent diabetic state in the dog, are to be explained by an antidiabetogenic hormone, then it should be possible to demonstrate the antibodies in the serum of the dog at a time at which the diabetic condition commences to disappear. In the last report mention was made of the fact that results had been obtained indicating that this indeed is the case. For example, two animals were given two intraperitoneal injections per day of a crude saline extract of beef anterior pituitary gland. Each of these injections corresponded to 20 grams of fresh gland, and was increased by the equivalent of 10 gms. on the third, sixth and ninth days, etc. Evidence of diabetogenic action was obtained on the third day, when the fasting blood glucose levels increased above normal and reached a maximum of 340 mg.% on the sixth day. This blood sugar level dropped suddenly to normal on the tenth and twelfth days, which was in accordance with the results which we have previously obtained with many animals. At this stage the animals were bled from the carotid artery, the blood being collected in sterile containers. The sera so obtained, when tested against the crude extract, revealed the presence of antibodies against pituitary in addition to those specific to ox protein. The actual titres obtained were 1/140 and 1/180 respectively, which, although low, are considered sufficiently high to explain the insensitivity to the extract. The second condition which must be fulfilled is the demonstration of power of serum from such an animal to protect another animal against the diabetogenic extract. This has also been investigated in two

ways. Fifty mls. of immune serum were injected intraperitoneally into a dog sixteen hours prior to the injection of 10 mls. of crude extract. It has already been shown that the injection of such an amount of extract will result in an increase in the blood sugar level, quite a characteristic curve being obtained. The blood sugar response to the injection of extract following the immune serum was followed, but a negative result obtained. However, since this serum contained antibodies, and moreover was capable of providing protection against the diabetogenic extract in rats, this failure is almost certainly due to the small volume of serum injected in comparison to the total body fluid of the animal. That the serum may be expected to give protection can be gauged from the results of two other experiments which were carried out. In these the blood glucose rise following 10 mls. of extract was determined. Ten mls. of this extract were mixed with 20 mls. of immune serum, and the mixture kept at 0° for 24 hours. The intraperitoneal injection of this mixture did not result in any alteration in blood glucose level. Because of the shortage of serum and the temporary cessation of Ennor's work, further experiments have not been carried out, but it would appear that the existence of an anti-diabetogenic hormone is reasonably well established. These experiments were carried out in collaboration with Dr. Singer.

Fat Metabolism.

Further work on the influence of anterior pituitary extracts on fat metabolism has been carried out. In general the results referred to in previous reports have been extended. With regard to the metabolism of isolated slices, perhaps the most interesting result is the fact that the oxygen consumption of the fatty livers is not significantly different from that of the normal livers. This is in marked contrast to the oxygen consumption of the livers of animals treated with carbon tetrachloride and phosphorus as referred to in the previous report. From the rate of production of ketone bodies, it is clear that fat in the livers of anterior pituitary-treated animals is being rapidly oxidised. Furthermore, the oxidation of large amounts of fat demands increased usage of oxygen. This increased usage of oxygen apparently is not reflected by the increased oxygen uptake, as is the case of phosphorus and carbon tetrachloride fatty livers. It would appear that the oxygen is made available for the oxidation of fats at the expense of that normally used for the oxidation of other substances.

In the last report mention was made of experiments which had been conducted which showed that the fat in the liver, after

injection of anterior pituitary gland extract, disappears five to seven days in spite of continued injection of the extract. This effect appeared to bear a close analogy to the disappearance of diabetic symptoms in the dog in spite of the continued injection of the extracts, and, as a working hypothesis, it was postulated that the appearance of antibodies were responsible for this effect. In order to test this, hutch rabbits were injected intravenously with extracts prepared with dried beef anterior lobes. The amounts of extract given in all cases were 1 ml. daily for the first three days, and thereafter 1 ml. every third day until the antibody content of the serum was considered sufficiently great. The titre of the immune serum that was used for the experiments to be described was 1 in 320, and prior to the injection into guineapigs all antibodies specific to ox protein were removed by absorption with ox serum. The animals used were well fed, male guineapigs, body weight about 300 grams. The injection of normal dog serum or immune serum produces no demonstrable effect either upon the fat content of the liver or upon the oxygen uptake and ketone body production of liver slices. Animals receiving one injection, equivalent to 0.5 gms. of anterior pituitary gland; exhibited a liver fat content of about 12 gms. per cent. eighteen hours after the injection, as compared with the normal fat content of 5 to 6 grams per cent. The injection of anterior pituitary extract is thus followed by increase of hepatic fat which still occurs if normal serum is given prior to the injection of the extract. If, however, immune serum is substituted for the normal serum the injection of anterior pituitary extract does not result in any change of the liver fat content. Complete protection against the metabolism effect is thus afforded. The metabolic behaviour as determined in the manner already referred to in experiments with carbon tetrachloride and phosphorus is normal in respect to those carried out in the absence and presence of added substrate. There is, however, some indication that animals treated with immune serum show evidence of increased rates of production of ketone bodies. Experiments have also been carried out using extracts which have been absorbed with crude extract, and here the protective power of the sera has been lost. The conclusions to be drawn from this series of experiments, which, although complete in themselves, have not yet been published, are:—

1. The phenomenon, described by Best and confirmed by Ennor, can be adequately explained on the basis of the formation of antibodies against pituitary fractions which combat the fatty liver producing effect of the anterior pituitary extract.

2. By the injection of immune serum it is possible to inhibit all transference of fat from the depots to the liver following subcutaneous injection of anterior pituitary extracts. It has been shown that this effect is due to a true antigen-antibody reaction.

Publications.

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

"Insulin Inactivation by Sulphydryl Compounds," "Australian Journal of Experimental Biology and Medical Science," vol. XX, 15, 1942.

A. B. CORKILL and P. FANTL:

"Carbohydrate Metabolism," "Australian Journal of Science," 5, 8, 1942.

A. H. ENNOR and E. SINGER:

"Serological Protection Against the Diabetogenic Substance of the Anterior Pituitary Gland," "Journal of Endocrinology," 3, 118, 1942.

A. H. ENNOR:

"Oxygen Consumption and Ketone Body Production in the Carbon Tetrachloride and Phosphorus Fatty Livers," "Australian Journal of Experimental Biology and Medical Science," XX, 73, 1942.

A. H. ENNOR:

"Iodine Numbers of the Liver Lipoids in Experimental Liver Damage," "Australian Journal of Experimental Biology and Medical Science," XX, 205, 1942.

P. FANTL:

"The Sterol Fraction of Australian Marine Mollusca," "Australian Journal of Experimental Biology and Medical Science," XX, 55, 1942.

P. FANTL, M. NOEL ROME and N. F. NELSON:

"Factors Controlling Glucose Formation in the Liver," "Australian Journal of Experimental Biology and Medical Science," XX, 121, 1942.

P. FANTL and M. NOEL ROME:

"The Breakdown of Commercial Starches by Muscle Extract," "Australian Journal of Experimental Biology and Medical Science," XX, 187, 1942.

Laboratory Tests on Patients with Jaundice.

In June, 1942, the Medical Staff of the American Fourth General Hospital appealed to us for assistance in the investigation of an outbreak of jaundice amongst their troops. They particularly desired that the Hanger cephalin-cholesterol flocculation test should be used to assist in the diagnosis and control of their cases. Mr. Douth, in association with Major W. Read, of the United States Army, has carried out tests in the Institute on an extensive series of cases. In all, 341 tests were carried out on 147 cases. The results show that the test was strongly positive in the acute symptomatic stage of the disease. The intensity of the reaction decreased as the clinical condition improved, and the test proved of value by indicating when a man might safely be returned to duty.

One aspect of the cephalin-cholesterol flocculation test which has caused difficulty both in the Institute and also in American laboratories is the tendency of some samples of cephalin to give emulsions which are flocculated by the sera of normal individuals. We have found that "ripening" of the cephalin by exposure to light and air for a period of at least six weeks overcomes this difficulty. Recent overseas publications confirm this finding.

The opportunity was taken, while carrying out the tests mentioned, to study the results obtained on the sera of patients suffering from obstructive jaundice. The very limited number of tests shows that the reaction affords a valuable means of differentiating this type of jaundice from that caused by hepatitis.

A paper dealing with various aspects of the joint work was read by Major Read at a meeting of the College of Physicians. Suitably revised, this material will be used as the basis for a publication on the subject in the near future.

Further co-operation is being extended to other members of the Staff of the Fourth General Hospital by the performance of Hanger flocculation tests on the sera of their patients.

BACTERIOLOGICAL SECTION.

Staff:

Dr. E. Singer.

Miss Mary H. Petherick.

Hormones of the Anterior Pituitary Gland.

This work, which was discussed in the last report, has been temporarily abandoned because of Mr. Ennor's transference to the Department of Munitions. So far the results found show that anti-sera, produced in rabbits by immunization with the partially purified diabetogenic fraction, can protect partially depancreatized rats against the glycosuric action of anterior pituitary extracts. Furthermore, the glycosuria occurring when excessive amounts of pancreatic tissue are removed can be suppressed by the anti-serum above referred to.

The different hormonal fractions have, serologically, sharply distinct specificities. Absorption of the crude anti-serum with different, highly purified hormonal fractions showed that the various antibodies exhibit different activities in respect of the anti-diabetogenic action of such sera.

Studies in Diphtheria.

It has been known for some time that the toxin production of diphtheria bacilli is inhibited when a medium containing iron is used. The explanation hitherto given for this phenomenon was that diphtheria bacilli require iron for their metabolism, and that toxin is produced to take the place of some other compound if iron is absent.

Dr. Singer and Miss Petherick have not found any experimental evidence in favour of this assumption.

It could be shown that minute amounts of iron have a very marked effect on infection with living bacilli, i.e., 1 microgram of iron in the form of ferrous sulphate prevents practically all pathological changes following the intradermal injection of living diphtheria bacilli in guineapigs. The necrosis, which usually develops within 24-48 hours, can be prevented, the infiltration is either absent or very much reduced, and the only symptom observed is usually a slight redness of the skin. The bacilli, however, are not killed, and can be cultivated from the site of infection without any difficulty. Other iron compounds have a similar action, the only difference being the amounts required, i.e., ferric ammonium citrate 15 micrograms, ferritin

and haemoglobin 5 micrograms, potassium ferricyanide and potassium ferrocyanide approximately 500 micrograms. Finally, it has been shown that copper and zinc compounds have a similar action.

The first definite indication of the real nature of this phenomenon was gained in experiments with diphtheria toxin. It could be shown that iron compounds not only inhibit the pathological changes which occur after infection with living diphtheria bacilli, but also those following an intradermal injection of preformed diphtheria toxin. Of a number of iron compounds tested, only ferrous sulphate and cytochrome C. were found to be active. It is significant that cytochrome has this activity, since this substance is a very important constituent of the cells. It is intimately connected with oxidation mechanisms, and therefore of primary importance for maintenance of the normal cellular metabolism.

It was therefore very probable that the destruction of diphtheria toxin in the living animal is somehow linked with oxidation and reduction mechanisms, and further experiments were performed with the purpose of testing this hypothesis.

Such experiments could not be performed "in vivo" because conditions in the living animal cannot be varied sufficiently, any interference with the oxidation mechanisms resulting in death of the animal. Recourse was had to "in vitro" experiments, which were arranged in the following way. A dilution of diphtheria toxin was made in phosphate buffer, and the required additions made. After approximately five hours, the remaining toxin was titrated by intradermal injection on guineapigs.

By this method it was found that iron, copper and zinc compounds have no, or at the best only a very slight, action on diphtheria toxin, but that destruction became very marked if redox substances were present. The latter, however, required to be activated by small amounts of iron, copper or zinc compounds.

This detoxication of diphtheria toxin is not to any great extent dependent on the reaction of the mixture. The same degree of detoxication could be observed if buffers ranging from pH 7.5 to 6.5 were employed. Other experiments were performed with inactivated rabbit serum of pH 7.8 to 8.0, and the same detoxication occurred. Ascorbic acid, adrenalin and glutathione were found effective in concentrations which can be regarded as physiological.

This type of experiment gives, of course, no information on the mechanism of destruction of diphtheria toxin in the living

animal, and a special study will have to be made to solve this problem. Nevertheless, the principle will, in all probability, be the same, only the type of metal compounds and the redox substances concerned varying.

The importance of these studies can be stated as follows:—

Detoxication of diphtheria toxin in the living animal is intimately connected with the oxidation-reduction mechanism of the cell. Cytochrome, and possibly other physiologically active iron compounds, play an important part. Whether copper and zinc salts are active under conditions prevailing in the living animal will have to be further investigated, but as it is known that certain copper and zinc proteins have very strong and important biological activities, it is very likely that these enzymes are in some way concerned.

Will it be possible to synthesize a chemotherapeutic substance with antitoxic activity? Predictions of such a nature have to be made with the utmost caution, but certain facts justify a rather optimistic outlook. In direct contrast to the chemotherapy of protozoal or bacterial infections, the compounds which could be used in attempts at antitoxic chemotherapy would not need to be "poisonous" substances in the ordinary sense of the word. They would not have to attack a specific grouping in the causative agent of the disease, as, for instance, arsenic attacks the sulphhydryl groups in the protoplasm of the trypanosome or sulphanilamide inhibits the utilization of para-amino-benzoic acid by bacteria. On the contrary, such a compound would increase the activity of a normally occurring detoxication mechanism of the living body.

Further, the metals which were found to be active, i.e., iron, copper and zinc, are not such severe poisons as other metals used for chemotherapeutic purposes (arsenic, antimony, bismuth, gold, mercury).

The principle of detoxication of bacterial toxins as outlined for the diphtheria toxin will probably be applicable for all, or at least most, of the bacterial toxins. Preliminary "in vitro" experiments performed by Dr. Singer and Miss Petherick have shown that staphylococcal toxin, which shows not the slightest similarity to diphtheria toxin, can be detoxified in the same way.

Blood Transfusion Studies.

The production of immune serum suitable for determination of the Rh factor in human blood was undertaken with one

particular case of transfusion accident in mind. As well known, the production of suitable serum is very difficult, and depends to a large extent on the animal used for immunization, only one out of ten on an average giving a serum suitable for clinical application. A further complication has been that the Institute had no Rhesus monkeys available, and it was only through the kind collaboration of Mr. Kendall, Director of the Zoological Gardens, who permitted the bleeding of some monkeys, that the production of the serum could be undertaken at all.

Notwithstanding these difficulties, a serum was produced, and has been applied to the study of several cases.

An interesting case of transfusion accident which occurred in the private practice of Dr. John McLean has been studied and reported in the "Medical Journal of Australia." As far as could be ascertained, this is the first case of transfusion accident due to isoimmunization of the patient against blood factor N by a previous transfusion.

Vibrio Septique Toxin.

Dr. Singer was asked by Dr. Morgan, Director of the Commonwealth Serum Laboratories, to collaborate in the question of the production of a potent vibrio septique toxin. Experiments with different media and under different conditions of growth were performed, and a report submitted to the Commonwealth Serum Laboratories.

Publications.

MARY H. PETHERICK:

"Studies on Natural Resistance. 1. The Relative Resistance of Guineapigs and Mice to Diphtheria Toxin," "Australian Journal of Experimental Biology and Medical Science," XX, 263, 1942.

MARY H. PETHERICK and E. SINGER:

"The Influence of Metals on Diphtheria Intoxication," now being prepared for publication.

"The Biological Detoxication of Bacterial Toxins," in the press.

E. SINGER:

"Studies on the Value of X-ray Therapy in Experimental Gas Gangrene Infection," "Medical Journal of Australia," 4th July, 1942, page 1.

"Serological Protection Against Arsenic Compounds," "Australian Journal of Experimental Biology and Medical Science," XX, 209, 1942.

"Isoimmunization Against Blood Factor N," "Medical Journal of Australia," in the press.

ROUTINE CHEMISTRY DEPARTMENT.

Staff:

Miss J. P. Marks.

Miss R. Wysokier.

The department has been correlating the results of fractional test meals with the history, X-ray and follow up of the patients. While often helping to confirm the diagnosis, in many cases test meals have been of no assistance. In all 120 cases have been examined.

Test meals are usually typical in cases of advanced carcinoma, although in the earlier cases, the result of the test meals are often not helpful in arriving at a diagnosis. The same applies in cases of ulceration, but here the test meal seems to alter with the amount of healing present.

Some cases showed hyperchlorhydria, with no X-ray or other proven evidence of ulceration or other abnormality, and the same is true of some cases of achlorhydrias. The usefulness of histamine as a stimulant to free HCl has been proved, some patients showing achlorhydria after a gruel meal, but free HCl after an injection of histamine.

Prothrombin estimations have been carried out on patients in whom an insufficiency of this blood constituent was suspected. Where a decreased blood prothrombin content was found, usually in cases of obstructive jaundice, Vitamin K was administered in oily solution by injection. This usually occasioned a dramatic rise of the prothrombin content of the blood. In patients with severe parenchymatous liver damage no rise was observed.

The estimation of sulphapyridine in both blood and C.S.F. has been carried out on numerous cases in order to help regulate the dosage of the drug.

The volume of work in the routine department remains very steady, in spite of continued efforts to curtail various tests.

Glassware and chemicals are still difficult to obtain, and various adjustments have had to be made.

During the year ended 30th December, 1942, the following tests have been carried out for the Hospital:—

Blood Urea Estimations	487
Urinary Protein Estimations	269
Urea Concentration Estimations	268
Urea Clearance Tests	266
Blood Sugar Estimations	366
Blood Sugar Curves	268
Blood Sugar Curves for R.A.A.F.	104
Benedict Tests	463
Benedict Tests for R.A.A.F.	104
Acetone Tests	463
Acetone Tests for R.A.A.F.	104
Cerebro-spinal Fluid Examinations	805
Lange Colloidal Gold Curves	53
Basal Metabolic Rate Estimations	99
Basal Metabolic Rate Estimations for R.A.A.F.	6
Fouchet Tests	37
Van den Bergh Tests	39
Benzidine Tests	52
Pyramidon Tests	73
Diastase Tests	42
Blood Calcium Estimations	13
Fractional Test Meals	385
Blood Chloride Estimations	5
Bile in Urine Tests	50
Miscellaneous	182
	5,003
Electrocardiographs	507
	5,510

The financial statement for the year is appended.

A. B. CORKILL,
Director

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

Statement of Receipts and Payments from 1st January to 31st December, 1942.

RECEIPTS	PAYMENTS.
To Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions	By Balance at 31st December, 1941
£6,484 9 2	£1,455 5 9
„ Grant, Department of Health	„ Medical Salaries
794 10 6	£2,666 3 11
„ Donation, Felton Bequests Committee	„ Other Salaries and Wages 4,195 2 6
100 0 0	6,861 6 5
„ Interest—	„ Drugs, etc.
Australian Consolidated	238 15 9
Loan	„ Instruments and Glass-
£637 10 0	ware
Grain Elevators Board 93 15 0	201 6 11
731 5 0	„ Special Maintenance
„ Proceeds of Sale of—	568 17 11
Apparatus	„ Fuel and Lighting
4 3 6	99 15 11
Monographs	„ Insurance
9 5 5	36 2 1
Vaccine	„ Repairs
1 11 6	57 3 4
Sundries	„ Library
82 15 6	132 6 10
97 15 11	„ Printing, Stationery and
„ Medical Fees	Postage
238 13 7	59 16 4
„ Refund of Pay Roll Tax	„ Travelling
12 14 0	15 3 6
£8,459 8 2	„ Monograph
„ Balance	6 1 7
1,419 0 10	„ Sundries
£9,878 9 0	146 6 8
	1,561 16 10
	£9,878 9 0
Investment—	
Grain Elevators Board Inscribed	By Balance at 31st December, 1942
Stock	£1,419 0 10
£2,500 0 0	

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

(Signed) **FLACK & FLACK,**
Chartered Accountants (Aust.),
Hon. Auditors.

Melbourne, 17/5/43.