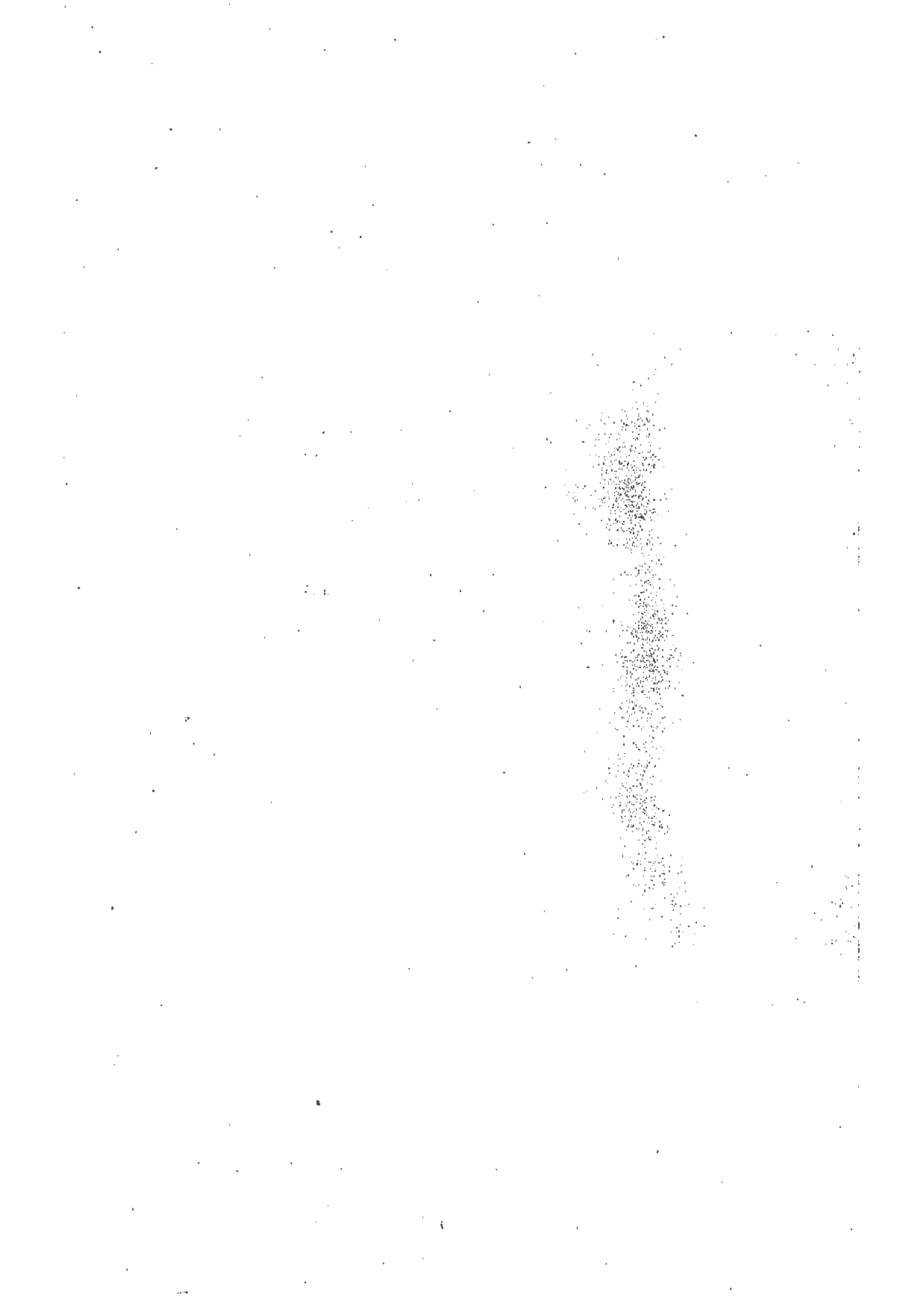
THE THOMAS BAKER, ALICE BAKER, AND ELEANOR SHAW MEDICAL RESEARCH INSTITUTE

FOURTEENTH ANNUAL REPORT

1939-40



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

ALFRED HOSPITAL, PRAHRAN, MELBOURNE.

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A. Basil Corkill, M.B., B.S., D.Sc. (Melb.), F.R.A.C.P., Director.

E. Singer, M.D. (Prague), Bacteriologist.

P. Fantl, D.Sc. (Vienna), Organic Chemist.

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E. Haab, Secretary to Director, and Librarian.

Full-time Workers under the National Health and Medical Research Council:

A. H. Ennor, M.Sc., Physiological Research.

Charlotte M. Anderson, M.Sc., Physiological Research.

Routine Hospital Work:

Jean P. Marks, Ph.C., Dip. Biochem. Analysis (London), Biochemist.

Jean C. Tolhurst, M.Sc., Bacteriologist.

A. H. Hyams, Bacteriologist (Hospital employee).

Ona M. C. King, B.Sc., attached to the Asthma Clinic.

Consulting Bacteriologist.

W. J. PENFOLD, M.B., CH.M. (EDIN.), D.P.H., B.HYG. (DUNELM), M.R.C.S (ENG.), L.R.C.P. (LONDON).

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The Director's Thirteenth Annual Report

TO THE TRUSTEES

of the

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

30th April, 1940.

Gentlemen,

Since my last report to you the scientific personnel of the Institute has been added to by the arrivals of Drs. Singer and Fantl, to take up the positions of research bacteriologist and organic chemist respectively.

Dr. Singer, who was appointed on the recommendation of Sir Henry Dale and Sir John Ledingham, was formerly Professor of Bacteriology at the University of Prague and, although his bacteriological researches have covered a very wide field, he has during recent years specialised in chemotherapeutic investigations. Originally it was intended that Dr. Singer should develop a chemotherapeutic unit at this Institute, but owing to the war and other conditions, this plan has had to be modified. Chemicals and apparatus usually obtainable from the Continent are now unavailable and, in addition, many of the bacteriological diseases on whch Dr. Singer has performed preliminary studies are non-existent in Australia. However, Dr. Singer has altered his plans, and details of his work will be found under the bacteriological section.

The Institute is greatly indebted to Associate Professor Davies, of the University of Melbourne, for his offer to assist Dr. Singer in his work, by the preparation of certain selenium compounds which he desires to test for chemotherapeutic properties.

In the past the laboratory has lacked facilities for carrying out research work involving organic chemistry. Personally, I have for some time felt the need for an organic chemist in my own researches on physiological and biochemical problems, and

the influence of organic chemistry is now being felt in certain fields of bacteriological research. To accommodate Dr. Fantl, the main biochemical laboratory has been subdivided to enable a small organic chemistry laboratory to be constructed. The alterations were so carried out as to allow for the inevitable expansion of Dr. Fantl's department.

In addition, we are gradually arranging facilities for carrying out micro-analyses on the various substances that Dr. Fantl is synthesising. In this connection we are greatly indebted to the National Health and Medical Research Council for supplying the Institute with a Kuhlmann micro-balance. We are still further indebted to this body for supporting the work of Mr. A. H. Ennor, M.Sc., and Miss C. Anderson, M.Sc., and for providing a Universal centrifuge with interchangeable heads. This centrifuge has now been in use for some months, and has been of great assistance to various workers.

The war situation has already affected the Institute to some extent. Apart from the difficulty of obtaining certain supplies, there was naturally, at the outset, some unrest, particularly amongst the younger members of the staff, because they were not able to carry out war work. However, the military authorities have full details of the personnel of our staff with their individual qualifications for special work, and on your behalf I have stated that should any special war problems arise the Baker Institute is prepared to place all its resources at the service of the Government.

The Institute is greatly indebted to Dr. F. G. Morgan, Director of the Commonwealth Serum Laboratories, for his ever ready and willing co-operation in supplying various bacteriological materials required for research work in this Institute.

It is pleasing to note that Max. Hume and Ken. Johnson, who joined the Institute staff as junior technicians, have appreciated the nature of their work to such an extent that they are now doing full-time University science and dental courses respectively.

Dr. Geoffrey Kaye, who has devoted so much time to the revision of "Practical Anaesthesia," the first monograph of the Baker Institute, has severed his connections with the Hospital and Institute, and is now abroad with the A.I.F.

On the 18th October, 1939, a meeting was held of representatives of the Hospital Board of Management and the Trustees of the Institute to discuss the future of the routine bacteriological department. It was pointed out that when the new pathological department was completed, and was under the

direction of Dr. R. A. Willis, it might be of mutual advantage to transfer the routine bacteriological service, now under the control of the Baker Institute, to Dr. Willis's department. It was finally decided that this transfer should be adopted on completion of the new building, and that the Baker Institute should still provide a routine biochemical service for the Hospital. It may be argued that a serious disadvantage of separating the routine from research bacteriology is that they would tend to be divorced, and would mutually lack co-operation. However, this possibility has been fully realised by Dr. Willis and myself, and I have his assurance that he will make available any material of interest to the research staff whilst, on the other hand, the research facilities of the Baker Institute will always be at the disposal of Dr. Willis's department.

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; l'Institut Pasteur d'Algérie; New York Academy of Medicine; Middlesex Hospital Medical School; South African Institute for Medical Research, Johannesburg; Lister Institute, London; Henry Lester Institute, Shanghai; the Medical Research Council, London; the Commonwealth Health Department; the Science Museum Library, London; the Walter and Eliza Hall Institute; l'Institut Sérothérapique de l'État Danois; School of Public Health and Tropical Medicine, Sydney; New York State Department of Health; Public Health Department, New South Wales; Turkish Central Hygiene Institute, Ankara, Turkey; Connaught Laboratories, University of Toronto, Canada; the London Hospital.

PHYSIOLOGICAL AND BIOCHEMICAL SECTION

Staff:

Dr. A. B. Corkill.	Mr. A. H. Ennor.
Dr. P. Fantl.	Miss C. M. Anderson.

Estimation of Sulphanilamide Compounds in Tissue Fluids.

Experiments on laboratory animals indicate that the therapeutic effects of sulphanilamide drugs are primarily due to the bacteriostatic action of such compounds. In addition, it has been shown that bacteriostatic action varies directly with the concentration of the drug. Accordingly clinicians, in order to

ensure that an adequate concentration of the particular sulphanilamide compound exists in the blood, are relying on the laboratory for such estimations. Recently the Institute was requested to carry out a large number of blood sulphanilamide estimations, and at first sight the method described by Werner seemed very suitable for routine work. However, when Dr. Fantl investigated the method, he found that, although it gives satisfactory values for free sulphanilamide, fallacious values are found for the combined form. This is due to the fact that the acetyl derivative is heated with trichloracetic acid, and Werner, apparently, has not realised that during the period of heating about 80 per cent. of the acid is destroyed. The decreased acidity of the solution influences the colour reaction with Ehrlich's reagent in the direction of giving values greater than the true ones.

After several attempts a new method for the estimation of combined sulphanilamide was evolved, in which α-naphthol was used. This compound in alkaline solution gives a red-coloured azo-dye with diazotized aromatic amines. The method has been applied to blood serum, cerebrospinal fluid, and urine, and found most satisfactory. Hydrolysis of the acetyl derivative is carried out with hydrochloric acid.

Studies have also been made on the distribution of M. & B. 693 in the blood of humans and rabbits. The interesting observation was made, whereas, following the oral administration of sulphanilamide, the parent substance of M. & B. 693, there is always a higher concentration of the drug in whole blood than in serum, yet with M. & B. 693 a higher concentration was invariably found in serum. It is difficult to explain why such closely allied compounds should, in the blood, exhibit such divergencies in distribution.

Again, it was found that the time of appearance of conjugated sulphanilamide in the blood stream, following oral administration, varies enormously in different individuals. It is uncertain whether such variations amongst normal persons depend on the conjugating power of the liver, or alterations in the renal threshold, for the excretion of the acetylated compound.

It is remarkable that acetylation seems to be limited to omnivorous and herbivorous animals. Thus the dog, largely a carnivorous animal, does not apparently possess the power to conjugate sulphanilamides. In view of the fact that the rabbit excretes an alkaline urine, whereas the dog has an acid one, some experiments were made concerning the influence of an alkaline diet on the excretion of the conjugated compound. Mice fed with a suspension of M. & B. 693 in Na_2CO_3 solution showed a greater excretion of the acetylated compound than controls fed with an aqueous suspension. This fits in with the work of James,

who found that mice given sodium acetate with sulphanilamide excreted more of the conjugated form. These findings suggest that a diet producing an alkaline urine may be of value in reducing the toxicity of drugs of the sulphanilamide group, and so eliminate undesirable symptoms following their administration.

Carbohydrate Metabolism.

Glycogen Breakdown in the Liver.

In conjunction with other work concerning the anterior pituitary gland, it was decided to investigate the action of pituitary extracts on various enzyme systems, including liver amylase. However, several publications recently appearing have thrown considerable doubt on the existence of a physiologically important amylase system in the liver, and investigations carried out by Dr. Fantl and Miss Anderson confirm this view. Several investigators have described an amylase system in liver, but the experiments were carried out under conditions in which the liver cells were damaged, i.e., liver brei or extracts.

Experiments here were made on intact and minced liver incubated at 37° C. Estimations of glycogen, total reducing substances, maltose and glucose showed that in the case of the undamaged liver, the sole end-product of glycogen breakdown was glucose and occasionally traces of maltose. In the minced tissue, however, maltose appeared regularly in small amounts, but again glucose formed the bulk of the reaction products. The figures found showed that the loss of glycogen was balanced by the formation of fermentable sugars. The conclusion must therefore be drawn that under physiological conditions liver tissue does not exhibit amylase activity, but damage to the cells, as by mincing, produces a slight degree of such activity. These results agree with the views of Willstätter and Rohdewald, that the liver contains a desmo-amylase bound to the cellular protoplasm and only apparent after disintegration of the cells. This, together with the findings of Dr. Fantl and his collaborators, demonstrates that amylase does not play a physiological role in the breakdown of liver glycogen.

In association with this investigation, studies have been made on the relation of phosphorylation mechanisms to glycogen breakdown. Dr. Fantl and his collaborators have extended the work of Cori and Kiessling, and in particular the influence of varying concentrations of sodium fluoride and inorganic phosphate on the end-products of glycogen breakdown has been studied. If liver pulp is incubated with inorganic phosphate at

pH 7.2 and up to a concentration of M/30, the sole end-product of glycogen breakdown is glucose. No influence on this course of events could be observed by the addition of M/200 sodium fluoride. With stronger phosphate solutions, however, say, M/10 to M/4, the reaction products now contain glucose monophosphate, but even in this case the mixture contains a preponderance of glucose.

Experiments designed to inhibit phosphatase activity in the liver reaction mixture showed that M/200 sodium fluoride was without effect, but with concentrations in the region of M/10 or M/5 an inhibitory action was observed. The decrease in inorganic phosphate was greater than in control experiments, but the reaction products still consisted of a mixture of glucose phosphates and glucose in the ratio of 1 : 1.

In Dr. Fantl's experiments it was found that the greater uptake of phosphate in the presence of sodium fluoride was not due solely to the formation of hexose monophosphate. At first it was thought that hexose-di-phosphate might be formed, a phenomenon which Kalckar observed in kidney extracts to which sodium fluoride and glucose were added, but no similar reaction was observed in liver pulp. The analytical data obtained in the experiments described were largely dependent on fermentation tests, but checks that were made showed that α-glucose-1-phosphate and glucose-6-phosphate were not fermented by yeast under conditions where glucose was completely fermented. In addition, it was found that 60 per cent. of glucose-6-phosphate is fermented under conditions where 100 per cent. maltose is fermented.

The slow rates of fermentation of glucose phosphates are remarkable since they have been isolated from yeast, and a more rapid fermentation would be expected if they were activated glucose derivatives. Since it has been shown that amylase does not function in the rabbit's liver in the conversion of glycogen to glucose, and that even with high concentrations of sodium fluoride and inorganic phosphate, glucose and glucose monophosphates are still formed in the ratio of 1 : 1, it is believed that either liver phosphatase cannot be inhibited under these conditions or that it is not concerned in the formation of glucose. In this case the monophosphates must be regarded rather as stabilisation than true intermediate products.

Sex Hormones.

Early in the year it was intended that the laboratory should collaborate with Mr. Fowler in the investigation of cases of

virilism from his Clinic. Preliminary reports from other institutes had suggested that in all such conditions there was an excessive excretion of androgens in the urine. Later, more carefully controlled investigations, particularly by Callow, have shown that the average excretion of androgens is, apart from frank cases of tumours or hyperplasia of the suprarenal cortex, within normal limits, and accordingly this investigation has not been continued.

Studies have, however, been made on the estimation of urinary pregnandiol, a compound which is believed to be a metabolic product of progesterone, the hormone of the corpus luteum. Previously the method used for the estimation of pregnandiol has been the gravimetric one of Venning, and owing to the necessity for frequent recrystallisations considerable losses are involved. A method has been evolved by Dr. Fantl, in which glycuronic acid determinations are carried out in the first precipitate. The estimation is a colorimetric one, and can be used to estimate very small quantities of pregnandiol glycuronidate, and it is planned to investigate the excretion of this compound in disorders of menstruation and pregnancy.

Sulphydryl Compounds and Insulin Action.

Inactivation of insulin in vitro, by sulphydryl compounds such as cysteine and glutathione (GSH), has been demonstrated by several workers, and in addition, it has been claimed that in the intact animal the hypoglycaemic action of insulin is decreased by the subcutaneous injection of cysteine or GSH. Levine and his co-workers have sought to correlate the sulphydryl content of the tissues with insulin sensitivity, and state that animals which are hypersensitive to insulin have decreased amounts of GSH in the liver. This view seemed at variance with some observations previously made in this Institute, and Mr. Ennor and I have carried out investigations on this problem. Mr. Ennor has already shown that animals treated with crude saline extracts of the anterior pituitary gland, and known to possess a resistance to the hypoglycaemic action of insulin, have definitely decreased values for liver GSH. We have made observations on rabbits rendered hypersensitive to insulin, and in all instances the values for liver GSH were within normal limits. The animals used were adrenalectomized, fasted or injected with ergotoxine. Again, young rabbits suffering from a mild degree of diphtheria toxaemia, and resistant to the hypoglycaemic action of insulin, showed no increased GSH content of the liver. In our opinion, therefore, there is no connection between liver GSH and insulin sensitivity.

various anaesthetics. These observations have been extended to the human being subjected to operation under anaesthesia, and as an index of the effect produced by anaesthesia and operative trauma the normal content of diphtheria antitoxin in human blood was chosen.

Some thirty sera were tested before and up to 48 hours after operation. It was found that the titre of antitoxins decreased in at least 50-75 per cent. of the patients. The number of sera tested is not large enough to make any dogmatic statements, but it appeared that a high initial titre of antitoxin was less sensitive against those factors tending to produce shock than a low one.

In the same sera total nitrogen, total globulin and, in some instances, pseudo-globulin were determined. No change was found in any of these constituents. The drop in antitoxin content cannot be explained therefore by loss of globulin, changes in the proportion of serum constituents or some similar factor. But some actual change, either in the nature of globulin or in the avidity of the antitoxic group, must occur.

Active Immunization Against Gas Gangrene and Tetanus.

The investigation of the combined tetanus alum-precipitated toxoid and B. welchii toxoid reported in the last report has been continued by Dr. Penfold and Miss Tolhurst, and satisfactory results have been obtained in guinea-pigs. Immunised animals have withstood 2000 minimum lethal doses of tetanus toxin, and also several minimum lethal doses of living B. welchii culture. The immunisation of man with a mixture of the two toxoids has not yet been attempted.

Publications:

E. SINGER:

"Studies on Blood Preservation." In the press.

"A Note on the Treatment of Gas Gangrene with Sulphanilamide and Related Compounds." In the press.

H. M. BUTLER and A. M. HILL:

"Haemolytic Streptococcal Infections following Child-birth and Abortion. I. Determination of Virulence of Group A Strains." "Medical Journal of Australia," 17th February, 1940, page 222.

"II. Clinical Features, with Special Reference to Infections Due to Streptococci of Groups other than A." "Medical Journal of Australia," 2nd March, 1940, page 293. (This work was commenced before Miss Butler left to join the Bacteriological Department at the Women's Hospital, and is published from both Departments.)

ROUTINE BACTERIOLOGY

The usual investigations have been carried out, and two new media have been introduced for the culture of typhoid bacilli, namely, Wilson and Blair's bismuth sulphite medium (Difco), and Leifson's sodium biselenite medium. These have considerably assisted in the isolation of B. typhosus.

Thanks are expressed to Miss H. M. Butler, of the Women's Hospital, and Miss M. Phillips, of the Bacteriology Department, University of Melbourne, for grouping and typing of haemolytic streptococci.

During the year the following routine bacteriological work has been carried out for the Hospital:—

Wassermann Tests	1,738
Gonococcal Complement Fixation Tests	210
Blood Cultures	115
Sundry Cultures	585
Vaccines	198
Investigations for Typhoid and Dysentery	91
Agglutination Tests	50
Pneumococcal Typing	10
Sundry Smears	95
Examinations for Gonococci	1,842
Dark Ground Examinations	52
Pregnancy Tests	78
Cultures for detection of tubercle bacilli	44
Miscellaneous	4
Total	5,112

ASTHMA CLINIC

This Clinic was originally formed in 1925 at the suggestion of an Honorary Physician of the Hospital, who had been greatly impressed by the treatment of allergic conditions in America, particularly in the clinic of Coca. At the start Dr. Charles Sutherland undertook to control the medical aspects of the clinic, whilst the present writer assisted by providing and standardising certain extracts required for diagnosis and treatment. From the outset, and at the present, the clinic has been greatly helped by the Commonwealth Serum Laboratories, who have made available an increasing number of substances concerned in allergic states. From a small unit the clinic has gradually developed to the position where now, in addition to Dr. Sutherland, there are four clinical assistants, two female voluntary workers, and a part-time science graduate. Miss King, who occupies the latter position, spends half of her time in work for the clinic and the rest of her time with the Baker Institute.

The number of patients attending during the hay fever season has increased this year, and there has also been a greater demand for mixtures used in specific therapy. The clinic now also supplies mixtures to doctors desiring to treat their patients privately. The Vollmer patch test has been used in conjunction with either the Mantoux or von Pirquet test in cases of suspected sensitivity to tuberculin. Of 63 children tested, only thirteen positive reactions were found, and in general the patch test gave results just as definite as that of the Mantoux test.

Miss King, in the time allotted to the Baker Institute, has been investigating the cephalin-cholesterol flocculation test, as described by Hanger, for the differentiation of obstructive and hepatogenous jaundice. In addition, its value as a test of liver function has been studied. The sera from sixty normal humans mixed with the cephalin-cholesterol emulsion gave no flocculation or deposition over a 48-hour period.

Sera from patients with cholecystitis, cholelithiasis, cirrhosis, hepatitis, obstructive, toxic and catarrhal jaundice were tested. It was found that where the jaundice was purely obstructive in origin no flocculation occurred, but whenever there was reason to suspect an associated liver cell damage a positive reaction was obtained. As judged by the clinical condition of the patient, the severity of liver involvement appears to parallel the amount of emulsion deposition. The reaction seems to be of definite value in assessing hepatic damage.

The financial statement for the year is appended.

A. B. CORKILL,
Director.

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

Financial Statement for Year 1st January to 31st December, 1939.

RECEIPTS.						PAYMENTS.					
To Balance at 31st December, 1938				£278	10 3	By Medical Salaries				£2,825	19 8
„ Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions				6,784	9 2	„ Other Salaries and Wages				4,014	2 4
„ Grants—										£6,840	2 0
Alfred Hospital	£1,000	0	0			„ Drugs, etc.				365	9 4
Department of Health	929	5	0			„ Instruments and Glassware				627	0 4
				1,929	5 0	„ Special Maintenance				723	1 5
„ Donations and Bequests—						„ Furniture and Equipment				18	12 0
Felton Estate	£100	0	0			„ Fuel and Lighting				189	7 11
Dr. Hagen	5	5	0			„ Insurance				30	15 7
Mr. Seeley	2	2	0			„ Repairs				29	0 10
H. E. Owen Estate	1	1	0			„ Alterations to Building				335	10 0
				108	8 0	„ Library				240	4 7
„ Interest—						„ Printing, Stationery and Postage				93	10 6
Australian Consolidated Loan	637	10	0			„ Travelling				8	2 2
Alfred Hospital	30	6	9			„ Sundries				95	8 3
				667	16 9					2,756	2 11
„ Proceeds of Sale of Monographs				26	12 7					£9,096	4 11
„ Proceeds of Sale of Equipment and Serums				45	1 0	„ Balance				1,130	12 1
„ Proceeds of Sale of Reprints				4	15 3						
„ Medical Fees				386	19 0						
				£10,226	17 0					£10,226	17 0
To Balance at 31st December, 1939				£1,130	12 1						

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

FLACK & FLACK,
Honorary Auditors.

Melbourne, 25th June, 1940.

MONOGRAPH ACCOUNT.

To Receipts—					By Expenditure—				
1933-34	£108	5	10		1933-34	£108	5	10	
1934-35	124	13	0		1934-35	20	17	5	
1935-36	57	14	7		1935-36	10	6	5	
1936	4	12	6		1936	251	17	6	
1937	55	8	3		1937	25	13	6	
1938	44	1	6		1938	Nil			
1939	26	12	7		1939	Nil			
				£421 3 3					£417 0 8
					„ Credit Balance				4 2 7
				£421 3 3					£421 3 3

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