COMMONWEALTH OF AUSTRALIA.

REPORT OF THE COMMISSIONERS.

INTRODUCTION.

To His Excellency the Right Honorable JOHN LAWRENCE, BARON STONEHAVEN, *a member of His Majesty's Most Honorable Privy Council, Knight Grand Cross of the Most Distinguished Order of Saint Michael and Saint George, Companion of the Distinguished Service Order, Governor-General and Commander-in-Chief in and over the Commonwealth of Australia.*

MAY IT PLEASE YOUR EXCELLENCY :

We, the Commissioners appointed by Royal Letters Patent to inquire into and report upon the deaths and illnesses associated with the use of toxin-antitoxin preparation at Bundaberg, Queensland, during the month of January, 1928, including the causes of those deaths and illnesses have the honour to report as follows :—

After preliminary experimental work conducted at the Walter and Eliza Hall Institute at the Melbourne Hospital, your Commissioners proceeded to Queensland and heard evidence at Stanthorpe, Toowoomba, Bundaberg and Brisbane. In New South Wales evidence was heard at Sydney, and in Victoria at Melbourne. The witnesses examined numbered forty-eight (48) in Queensland, three (3) in New South Wales and fifteen (15) in Victoria, making a total of sixty-six (66) persons. Since returning to Melbourne extensive experimental investigations have been conducted at the Walter and Eliza Hall Institute.

NARRATIVE OF EVENTS.

In pursuance of the policy of free active immunization against diphtheria, advocated by the Commissioner of Health for Queensland throughout that State, and adopted by the Council of the City of Bundaberg, Dr. Ewing George Thomson, Medical Officer of Health of Bundaberg, began the inoculation of children with diphtheria toxin-antitoxin mixture in January, 1928.

The material used was taken from an india-rubber capped bottle purporting to contain 10 c.cm. of diphtheria toxin-antitoxin mixture. The bottle was one of thirty issued by the Commonwealth Serum Laboratories, Melbourne, to the Commonwealth Health Department, Brisbane, on the 29th November, 1927. It belonged to Batch 86 which had been made up on the 5th September, 1927, to contain in 1 c.cm. 0.8 L + dose of diphtheria toxin together with one unit of antitoxin. No antiseptic was present in the mixture.

The bottle was forwarded to Dr. Thomson on 6th January, by Medical and Surgical Requisites Ltd., Brisbane, who procured it on the same day from the Commonwealth Department of Health, Brisbane.

On receipt Dr. Thomson stored the bottle until it was required in an instrument cupboard in his surgery, where it was also kept during the periods between the inoculations which he made from it.

The first inoculations were given on the 17th January, 1928. At the Bundaberg City Council Chambers, in a room previously occupied by the City Engineer, Dr. Thomson gave six children the first dose of two minims.

On the 20th January, 1928, at the same place, eight children received their first injection of two minims.

On the 21st January, 1928, at his surgery, Dr. Thomson gave three children, including his own son, the initial dose of two minims.

On the 24th January, 1928, at the Council Chambers, six children who had received their initial dose on the 17th January, were given a second injection of four minims, and in addition another child received her first injection.

No untoward effects resulted from any of these inoculations.

On the 27th January, 1928, in the room at the Council Chambers, between 4.10 p.m. and 5.5 p.m., the eight children who had received the initial dose on the 20th January, received their second injection of four minims and in addition thirteen children received an initial dose of two minims.

Of these twenty-one children, eighteen became ill with symptoms of significant similarity during the night of the 27th January or the early morning of the 28th January. Eleven died during the 28th January, and one on the 29th January.

During the morning of the 28th January all these children except one, who was brought to Dr. E. T. K. Schmidt's surgery but was found to be dead on arrival, were seen by Dr. Thomson, Dr. L. D. McKeon, Dr. S. Robinson or Dr. I. C. Hains. Fourteen were admitted to the Bundaberg General Hospital where nine died, several soon after admission. One was admitted to St. Vincent's Hospital and died there and one died in his own home.

Dr. Hains, the Medical Superintendent of the Bundaberg General Hospital, at about 3 p.m on the 28th January, informed the Senior Sergeant of Police at Bundaberg of the deaths occurring at his hospital and intimated that he was not prepared to sign death certificates.

The Sergeant of Police reported to the Inspector at Maryborough who gave instructions that post-mortem examinations of all the bodies were to be made by Dr. E. T. K. Schmidt, the Government Medical Officer at Bundaberg.

While the medical men at Bundaberg appear to have done all that was possible in the circumstances, they were confronted by an emergency which taxed all their powers. The unavoidable absence of skilled specialist aid early on the 28th (at which time no outside help could have been available in any circumstances) and the heavy strain on the resources of the hospital staff in the treatment of so many very sick children, naturally made the clinical records somewhat incomplete.

It early became evident that it was desirable to obtain expert assistance. On becoming aware of the facts and in view of the responsibility of the City Council, the Mayor of Bundaberg at once attempted to get into communication with the Home Secretary (Queensland) to obtain if possible the services of an expert pathologist from Brisbane, who, he suggested, might travel up by special train that night or by aeroplane in the morning.

There was an unavoidable delay in conveying this request to the Home Secretary and the importance of sending expert assistance was not realized by the State Authorities, who evidently regarded the matter as the province of the Commonwealth and were anxious only to avoid overlapping. On learning the steps which the Director of Tropical Hygiene, Commonwealth Department of Health, Brisbane, proposed to take, the State Authorities considered that no further action by them was called for (See Appendix 9).

Consequently no pathologist was available until the 30th January when Dr. Richards of the Commonwealth Health Laboratory, Rockhampton, arrived at Bundaberg, and by then the only material available for autopsy was the body of one child who had been dead fifty-two and a half hours, during which time at the temperature of Bundaberg much post-mortem change must necessarily have occurred.

On the evening of the 28th and the morning of the 29th January, Dr. Schmidt had already carried out autopsies on eight bodies at times varying from four to twelve hours after death. He also performed the autopsy at which Dr. Richards was present. From three of the bodies he retained for subsequent investigation certain organs which were placed in spirit, unfortunately in a manner unsuitable for their satisfactory preservation. Consequently, much of the material was of little value. Dr. Richards preserved portions from one other body. Dr. Schmidt had had no special post graduate training in pathology, and Dr. Richards' training had not been specially directed to this branch of investigation, so that many important possibilities which required immediate investigation were overlooked.

The Director of Tropical Hygiene also sent Dr. G. A. Murray from Brisbane to Bundaberg, where he arrived on the 30th January to inquire into the circumstances of the tragedy from an administrative point of view.

Finally Dr. Tilling, on the 31st January, arrived on behalf of the British Medical Association and the Brisbane City Council and made further investigations which later proved of considerable value.

On receipt of the news of the tragedy, the Director-General of Health, Dr. J. H. L. Cumpston, gave instructions that all toxin-antitoxin of Batch 86 should be withdrawn and that no further issues of toxin-antitoxin should be made.

The remainder of the toxin-antitoxin mixture used by Dr. Thomson was handed over to the Mayor on the afternoon of the 28th January. Its subsequent history is dealt with later, (Appendix 18.)

On the 1st February, 1928, a Royal Commission was appointed to inquire into the fatalities at Bundaberg.

CLINICAL HISTORY OF THE CHILDREN INOCULATED ON 27TH JANUARY, 1928.

1. THOSE WHO RECEIVED THEIR FIRST INJECTION OF TWO MINIMS ON 27TH JANUARY, 1928.

Case 1—*W.F., Male—aged two years and six months.*—He had had scarlet fever three months and his mother had had diphtheria two months before. He received his inoculation soon after 4 p.m. on 27th January. He had two large evening meals, was in the best of spirits at 7 p.m. and went to sleep about 9 p.m. At 11 p.m. he woke his parents up " rattling his cot with the shivers." His body was cold and his head very hot. From 11.30 p.m. to 11.45 p.m. he vomited. He was given castor oil but no drinks, though he asked for water. He slept from midnight to 7.30 a.m. and was then found by his parents in an unconscious condition. He was apparently deeply cyanosed with a blotchy purple mottling of the skin of the body, with blue lips and eyelids and was breathing heavily. He was taken by car at about 8 a.m. on 28th January to Dr. Schmidt's surgery, but was found to be dead on arrival there.

Case 2—*F.B., male—aged nine years and six months.*—There was no history of diphtheria or other infectious disease. He was inoculated about 4.30 p.m. on 27th January, 1928, and there were no symptoms whatever until 29th January, 1928, when the site of inoculation became swollen and slightly painful. A day or two later an abscess formed and discharged pus.

Case 3—*G.B., male—aged five years and eleven months.*—He had had no history of diphtheria or other infectious disease, but suffered from " coeliac disease." He was inoculated at 4.30 p.m. on 27th January. His illness commenced at 10 p.m. with vomiting and diarrhoea and continued till 3 a.m. on 28th January, 1928. He was seen by Dr. Thomson at 8.30 a.m.—temperature 101°. At 11.45 a.m. he was admitted to the Bundaberg General Hospital—temperature 104°, pulse 120, respirations 40. An enema was given with a green offensive slimy result. At midday 4,000 units of diphtheria antitoxin were given intramuscularly. At 2 p.m. he was pallid and unconscious, with dilated pupils, temperature 102.8°, pulse 132 respirations 56. After 2.30 p.m. there were continuous convulsions and he was deeply cyanosed and vomiting green fluid. He died at 5.35 p.m. (See Appendix 8 for temperature chart.)

Case 4—*Monica S., female—aged two years and nine months.*—There was no previous history of diphtheria. She received her inoculation between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was between 9 and 9.30 p.m., with vomiting and diarrhoea all night. She was still conscious at 7 a.m. At 10.15 a.m. on the 28th January, 1928, she was seen by Dr. Thomson and admitted to the Bundaberg General Hospital. She was then unconscious with rolling eyes and glassy stare. The pupils were dilated though she had not yet received any atropine. There was deep blotchy cyanosis ; temperature 102.8°, pulse 160, respiration 70. At 11 a.m., 4,000 units of antitoxin were given intramuscularly. An enema was administered, with green offensive slime as result. She convulsed continuously, the convulsions being preceded by a cry, and vomited from time to time first green and later brown fluid. At 2 p.m., temperature 104°, pulse uncountable, respirations 92. She died at 3.15 p.m. (For temperature chart, see Appendix 8.

Case 5—*Maisie S., female—aged four years and eleven months.*—There was no previous history of diphtheria. She received her inoculation between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was between 9 p.m. and 9.30 p.m., with vomiting and diarrhoea until morning. She was still conscious at 7 a.m. on 28th January, 1928. At 10.15 a.m., she was seen by Dr Thomson and admitted to the Bundaberg General Hospital ; temperature 103.2°, pulse 180, respirations 60. She was then unconscious, with rolling eyes, glassy stare, and dilated pupils. There was a deep blotchy cyanosis. An enema was given, resulting in a green slimy offensive stool. At 11 a.m., 4,000 units of antitoxin were given intramuscularly. She had convulsions and vomited, at first green, but later brown-coloured fluid. At 2 p.m., temperature 105.2°, pulse uncountable, respirations 80. She died at 3.15 p.m. (For temperature chest, see Appendix 8.)

Case 6—*W.S., male—aged six years and six months.*—There was no previous history of diphtheria. He received his inoculation between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was between 9 and 9.30 p.m., with vomiting and diarrhoea until morning. He was still conscious at 7 a.m. on 28th January, 1928. He was seen by Dr. Thomson and admitted to the Bundaberg General Hospital at 10.15 a.m. ; temperature 104°, pulse 166, respirations 74.

An enema was given with green slimy offensive result, and 4,000 units of diphtheria antitoxin were given intramuscularly. It is doubtful if he was unconscious on admission, but he would not speak. He did not know his father at 11.30 a.m. The area round the site of inoculation was inflamed. There was projectile vomiting and cyanosis. (For temperature chart see Appendix 8.)

2 p.m.—Temperature 103°, pulse 144, respirations 74.
6 p.m.—Temperature 102.4°, pulse 160, respirations 70, twitching of face and shoulders.
10 p.m.—Temperature 100.6°, pulse 160, respirations 36.
11 p.m.—Temperature 100.8°, pulse 140, respirations 48.
12 p.m.—Pulse 144, respirations 38, retention of urine, restless and vomiting. Catheterized and 7 ozs. of urine were obtained.

29th January, 1928—

1 a.m.—Temperature 100.8°, pulse 136, respirations 32.
2 a.m.—Pulse 121, respirations 40, restless.
3 a.m.—Pulse 140, respirations 24, B.W.O. green fluid stool.
4 a.m.—Pulse 136, respirations 26, speech more rational.
6 a.m.—Temperature 98.6°, pulse 136, respirations 24.
8 a.m.—Pulse 124, respirations 28, B.W.O. voided urine with stool.
10 a.m.—Temperature 99°, pulse 140, respirations 32.
12 m.d.—Temperature 97.6°, pulse 114, respirations 32, restless.
2 p.m.—Temperature 97.8°, pulse 120, respirations 32, pain in left chest.
4 p.m.—Temperature 98.2°, pulse 120, respirations 32.
6 p.m.—Temperature 98.6°, pulse 116, respirations 30.
7 p.m.—Temperature 97°, pulse 128, respirations 30, voided urine, 1032—no albumin, no sugar. Vomited sour curds.
8 p.m.—Temperature 98°, pulse 130, respirations 30, coughing.
12 m.n.—Pulse 124, respirations 28, sleeping.

30th January, 1928—

12.30 a.m.—Temperature 98.2°, pulse 134, respirations 36.
2 a.m.—Temperature 97.4°, pulse 112, respirations 26, sleeping.
6 a.m.—Temperature 98.4°, pulse 120, respirations 24.

During this day the temperature remained about 98.4°, pulse 108 to 120 and respirations 26 to 32, and there was a definite pleuritic rub on the left side of the chest. About midday he complained of severe abdominal pain. This was relieved after an enema which yielded a dark green offensive stool. On this day Dr. Richards obtained a blood film which showed 91 per cent. polymorphs in the differential count.

On 31st January, 1928, the temperature, pulse and respirations remained about the same as on the 30th, and had returned to normal by the 2nd February. On the 6th, the area of inflammation at the site of inoculation had become hard and very painful, and on the 7th February temperature, pulse and respirations showed a marked rise doubtless due to this. On the 8th February, Dr. Hains evacuated an abscess from which Dr. Richards isolated a staphylococcus in pure culture.

Case 7—J.S.—male, aged eight years and one month.—There was no history of diphtheria. He received his inoculation between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was between 9 and 9.30 p.m. with vomiting and diarrhœa all night. He was seen by Dr. Thomson in the morning and admitted to the Bundaberg General Hospital at 10.15 a.m. on 28th January, 1928; temperature 103°, pulse 176, respirations 60. 4,000 units of diphtheria antitoxin were given intramuscularly. He was only semi-conscious on admission but knew his father at 11.30 a.m. An enema gave a green slimy offensive result. (For temperature chart, see Appendix 8.)

2. p.m.—Temperature 103°, pulse 148, respirations 68.
6 p.m.—Temperature 102.4°, pulse 172, respirations 52, very restless.
11 p.m.—Temperature 101.2°, pulse 140, respirations 48, restless and twitching.

29th January 1928—

1 a.m.—Temperature 101.2°, pulse 146, respirations 48, catheterized 11-ozs.: urine 1,034 normal.
2 a.m.—Pulse 126°, respirations 30, B.W.O., green slime with mucus.
3 a.m.—Temperature 101°, pulse 132, respirations 40, vomited offensive greenish fluid.
6 a.m.—Temperature 99.8°, pulse 140, respirations 28, more rational but vomiting green fluid.
9 a.m.—Temperature 103°, pulse 144, respirations 48, restless.
12 midday.—Temperature 98.4°, pulse 126, respirations 36, dark green slimy stool.

2 p.m.—Temperature 98°, pulse 128, respirations 40.
4 p.m.—Temperature 99.6°, pulse 140, respirations 24.

Later in this day he passed urine normally twice; Sp. Gr. 1,020, acid, normal. There was some cough and the pulse remained high. On the following day the temperature was a little above normal, the pulse ranged from 112 to 128 and the respiration rate ranged from 22 to 40. On this, the third day of the illness, Dr. Richards made a blood culture, which was sterile.

On 31st January, 1928, the temperature was almost normal, but the pulse varied from 102 to 112.

On 1st February, 1928, he was much better, taking nourishment well, and he remained well with only very slight pyrexia until 6th February, when his temperature rose slightly and the local swelling at the site of inoculation increased and became definitely tender. On the 7th, the abscess opened spontaneously and pus discharged from it. From this time the patient made an uninterrupted recovery, though his temperature and pulse rate were slightly above normal from time to time, and the arm discharged intermittently for some days.

Case 8—*N.W.—female, aged four years and seven months*, was said by the parents to have had diphtheria twelve months before. This was corroborated by Dr. Robinson but not confirmed by the records of reported cases. She received her inoculation on 27th January, 1928, about 5 p.m. and thereafter had no symptoms until about three days later when a swelling appeared at the site of the inoculation. On about 9th February, 1928, this broke down and discharged pus. This child and her sister were seen on 28th January, 1928, by Dr. Robinson who found them perfectly normal.

Case 9—*C.W.—female, aged eight years and five months.* had diphtheria about a year before. She received the first inoculation about 5 p.m. on the 27th January, 1928, and had no symptoms of illness until three days later when the arm at the site of inoculation became painful and tender. Pus discharged about 9th February, 1928.

Case 10—*V.T.C.,—male, aged five years and ten months.*—There was no history of diphtheria or any other infectious disease. He was inoculated at about 5 p.m. on 27th January 1928. Twenty hours later he had a temperature of 100° and a slight headache. There were no other symptoms. On 15th February, 1928, a small local abscess was opened on the arm at the site of inoculation and a staphylococcus was isolated in pure culture from the pus.

Case 11—*G.M.C.—male, aged seven years and three months.*—There was no history of diphtheria or any other infectious disease. He was naturally a nervous child and vomited readily if upset or frightened. He was inoculated at about 5.5 p.m. on 27th January, 1928. The onset of symptoms was at 11 p.m. with vomiting, great distress and helplessness. He could not sit up properly. The bowels were opened three times during the night with foul-smelling stools. He was also delirious. In the morning he was still conscious but was breathing in short gasps and was "very blue and white". Dr. Robinson saw him about 8 a.m. He was then in a very excitable state throwing himself about on the bed. He was cyanosed, temperature 103° and no pulse could be felt. The heart was "fluttering". He died soon after the doctor's arrival.

Case 12—*J.P. female, aged five years and nine months.*—There was no previous history of diphtheria or infectious disease. She was inoculated between 4 and 5 p.m. on the 27th January, 1928. The onset of symptoms was at 10.30 p.m. when she felt sick, vomited and had diarrhœa. On the morning of 28th January, 1928, she was seen by Dr. Thomson who had her removed to the Bundaberg General Hospital. On admission at about 10 a.m., temperature 102.6°, respirations 60. She was collapsed with an imperceptible pulse, was unconscious but not convulsing. The stools were green, slimy and offensive. 4,000 units of diphtheria antitoxin, were given intramuscularly. There was no cyanosis and her colour was waxy. At 2 p.m., temperature 103.4°, pulse 140, respirations 80. She died at 2.30 p.m. on 28th January, 1928. (For temperature chart, see Appendix 8.)

Case 13—*B.P.—female, aged six years and nine months.*—There was no history of diphtheria or other infectious disease. She was inoculated between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was soon after 10.30 p.m. with vomiting and diarrhœa. She was seen on the morning of 28th January by Dr. Thomson who had her removed to the Bundaberg General Hospital where she was admitted at 10 a.m. in a collapsed condition—temperature 103.6°, pulse 168, respirations 58. There was a green slimy offensive result from an enema. 4,000 units of diphtheria antitoxin were give intramuscularly. The pupils were dilated, the lids inflamed and a rash was observed on the body. She was delirious. (For temperature chart, see Appendix 8.)

2 p.m.—Temperature 103°, pulse 180, respirations 56.
4 p.m.—Temperature 102°, pulse 180, respirations 56.
6 p.m.—Temperature 102.6°, pulse 156, respirations 52.
10 p.m.—Temperature 100.2°, pulse 174, respirations 44.
12 midnight.—Pulse 140, respirations 40.

29th January, 1928—

She was catheterized at 1.30 a.m., and 7 ozs. urine were obtained; Sp. Gr. 1020, acid normal.

2 a.m.—Temperature 99.2°, pulse 108, respirations 28.

4 a.m.—Pulse 136, respirations 36, dark green stool with mucus.

6 a.m.—.Temperature 99.8°, pulse 100, respirations 26.

10 a.m.—Temperature 100°, pulse 104, respirations 28, very restless.

12 midday.—Temperature 98.8°, pulse 134, respirations 34.

2 p.m.—Temperature 99.6 °, pulse 136, respirations 32, voided urine.

8 p.m.—Temperature 99.8°, pulse 136, respirations 32.

During the night she was restless, but slept. She voided urine normally. By the early morning of the 30th, the rash was all over the body and hands and was extremely irritable. The temperature ranged between 99.8° and 101°, the pulse from 116 to 144 and the respirations from 26 to 36. Blood was taken in the evening for culture by Dr. Richards—result negative. The next day, 31st January, 1928, her condition had definitely improved. The temperature ranged from 98° to 99.8°, the pulse from 112 to 136, and the respirations from 24 to 28. The area on the arm at the site of inoculation was now definitely inflamed.

On 1st February, 1928, the temperature, pulse and respirations were about the same and she complained of a sore throat.

Her condition improved steadily though the local inflamation at the site of inoculation persisted. On the 13th February, 1928, Dr. Hains incised the abscess in the presence of the Commission who isolated a staphylococcus in pure culture from the pus. When seen on the 13th February, 1928, there was desquamation of the skin of the hands and feet.

TABLE 1.

SUMMARY OF ILLNESSES OF CHILDREN WHO RECEIVED THEIR FIRST INOCULATION OF TWO MINIMS OF TOXIN-ANTITOXIN MIXTURE ON 27TH JANUARY, 1928.

Cases in one family are indicated by bracketing together.

Time of Death recorded in hours after injection.

No.	Age.	Sex.	Time of Onset.	Symptoms.	Result.
1	Two and a half years	M.	7 hours ..	Shivering, vomiting (early), stupor—cyanosis, respiratory distress	Died 15 hours
2	Nine and a half years	M.	.. ..	None, abscess developed at site of inoculation	No illness
3	Five years and eleven months	M.	5½ hours ..	Early vomiting and diarrhoea. Pyrexia—collapse—pallor, unconsciousness, late cyanosis—convulsions and vomiting	Died 25 hours
4	Two years and nine months	F.	5 hours ..	Early vomiting and diarrhoea. Pyrexia, blotchy cyanosis, coma, convulsions, late vomiting	Died 23 hours
5	Four years and eleven months	F.	5 hours ..	Early vomiting and diarrhoea. Pyrexia, blotchy cyanosis, coma, convulsions, late vomiting	Died 23 hours
6	Six years and six months	M.	5 hours ..	Early vomiting and diarrhoea, pyrexia, stupor, projectile vomiting, cyanosis. Twitching and restlessness, retention of urine. Signs of pleurisy second day. Abscess at site of inoculation	Recovered
7	Eight years and one month	M.	5 hours ..	Early vomiting, diarrhoea, stupor, pyrexia. Recovered from severe symptoms second day. Later abscess at site of inoculation	Recovered
8	Four years and five months	F.	.. ..	None—later abscess at site of inoculation ..	No illness
9	Eight years and five months	F.	.. ..	None—later abscess at site of inoculation ..	No illness
10	Five years and ten months	M.	20 hours ..	Headache, slight pyrexia, later abscess at site of inoculation	Recovered
11	Seven years and three months	M..	6 hours ..	Vomiting, distress, slight diarrhoea, pallid, cyanosis, restlessness	Died 15 hours
12	Five years and nine months	F.	6 hours ..	Early vomiting and diarrhoea. Pyrexia, collapse, unconscious, no cyanosis, waxy pallor, no convulsions	Died 21 hours
13	Six years and nine months	F.	6 hours ..	Early vomiting and diarrhoea, pyrexia, collapse, dilated pupils—generalized rash on body. Restlessness and delirium. Recovery from severe symptoms in two days. Later abscess at site of inoculation	Recovered

Of these thirteen children, six were females and seven males. Three females and three males died. The average age of the group was 5.8 years. The average age of those who succumbed was 4.7 years. The average age of the survivors was 7.1 years. The illnesses in families suggest that age or possibly body weight influenced the result, for on the whole the younger children died and the older recovered. An exception to this is afforded by the C. children in which the elder child died. In the B. family the younger child had suffered from coeliac disease. The absence of illness on the W. children suggests a familial immunity.

Children who Received Their Second Inoculation of Four Minims on the 27th January, 1928.

Case 14—*K.B.—male, aged three years and two months.*—There was no history of diphtheria but he had been a carrier about a year before. There was no history of other infectious disease. He was inoculated on 20th January, 1928 and between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was at 9.30 p.m. with vomiting followed by diarrhoea, until 12 midnight, then only occasionally to 3.30 a.m. He was unconscious after 2.30 a.m. In the morning he was limp, with short quick breathing and cyanosis. Seen by Dr. Hains and admitted to hospital at 8.45 a.m.—temperature 103°, pulse 190, respirations 80. He was then cyanosed, unconscious, with glassy eyes, rolling stare and dilated pupils. The pulse was poor in volume. An enema was given and a green slimy offensive stool resulted. 4,000 units of diphtheria antitoxin were given intramuscularly. He convulsed almost continuously to 10.30 a.m. There was blotchy cyanosis and vomiting. He died at 11.45 a.m. (For temperature chart, see Appendix 8.)

Case 15—*E.B.—male, aged five years and nine months.*—There was no history of diphtheria or other infectious disease. He was inoculated on 20th January, 1928, and between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was at 9.30 p.m., with vomiting and diarrhoea until midnight, when he settled down with only occasional vomiting until 3.30 a.m. He was unconscious at 2.30 a.m. At 8.45 a.m. on 28th January, 1928, he was seen by Dr. Hains and admitted to the Bundaberg General Hospital; temperature 103.4°, pulse 170, respirations 72. He was then unconscious, deeply cyanosed, with glassy stare and dilated pupils. The pulse volume was poor. A green slimy stool followed an enema. 4,000 units of diphtheria antitoxin were given intramuscularly. From 9.15 a.m. he was convulsing continuously, appearing to have pain before the onset of convulsions and was very restless and cyanosed. The colour of the lips improved temporarily at 2 p.m. after administration of digitalis. At 2 p.m.—temperature 102.4°, pulse 118, respirations 48. He vomited frequently, first green and later brown material. The breathing became irregular and he died at 4.15 p.m. on 28th January, 1928. (For temperature chart, see Appendix 8.)

Case 16—*M.Br.—female, aged three years and six months.*—There was no history of diphtheria or other infectious disease. Her brother had just had diphtheria and came out of hospital on the 26th January, 1928 after three weeks there. She was inoculated on 20th January, 1928, and between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was with dry retching at 12.10 a.m. There was then no pyrexia. She had no diarrhoea at any time. At 6.30 a.m. she got out of bed and was able to walk out to the kitchen and ask for a drink of water. She was conscious until 9 a.m., when she was seen by Dr. Thomson and sent to the Bundaberg General Hospital. She was admitted there about 11.30 a.m. 4,000 units of diphtheria antitoxin was given before admission. Temperature 105.4°, pulse 196, respirations 64; then unconscious and cyanosed with a rash like a serum rash on the body. The pupils were dilated, the eyes rolling and the stare glassy. A green slimy stool followed an enema. She was convulsing almost continuously and her colour was very blue. There was twitching of the face before the onset of the convulsions. Later there was projectile vomiting. She died at 5.30 p.m. on 28th January, 1928. (For temperature chart, see Appendix 8.)

Case 17—*B.D.—female, aged one year and three months.*—Two other children in this family were in hospital with diphtheria on 20th January, 1928. She was inoculated on 20th January, 1928, and between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was about 1 a.m. on 28th January, 1928. She was then still asleep but feverish and somewhat later vomited. She was seen by Dr. Thomson at 2 a.m. (temperature 102.3°) and was sent to the Bundaberg General Hospital, where she was admitted at 3.15 a.m.—temperature 102.8°, pulse 134, respirations 64. There was definite inflammation at site of inoculation.

Her temperature ranged from 100 to 102.4° during the day, but the pulse and respiration rates were disproportionately rapid—120 to 180 and 32 to 60 respectively. The temperature remained above normal until 29th January, 1928, but the pulse and respiration rates were high for some days later. On 5th February, 1928 she was discharged from hospital apparently well, except for a rapid pulse rate but was re-admitted on 7th February, 1928, on account of an abscess in the arm at the site of inoculation. This was opened by Dr. Hains on 9th February, 1928, and

pus was evacuated from which a staphylococcus was isolated by Dr. Richards. The local infection at this stage caused no pyrexia though the pulse rate was still somewhat high. (For temperature chart, see Appendix 8.)

Case 18—*T.R.—male, aged five years and six months.*—There was no history of diphtheria. He was inoculated on 20th January, 1928, and again between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was at 9.30 p.m. He vomited for about ten minutes and then had diarrhoea for about an hour, He then became febrile. At 6 a.m. his temperature was 103°. At about 9 a.m. he was seen by Dr. McKeon who was in the house about two and a half hours. His general condition was good early in the morning, though he was irritable and feverish. Later he became worse and at 12.45 p.m. on 28th January 1928 he was admitted to the Bundaberg General Hospital. He was then unconscious, with blotchy cyanosis and dilated pupils—temperature 100.4°, pulse 116, respirations 20. An enema gave a green slimy offensive result. Soon after admission his face and hands were twitching, though he was not convulsing.

At 2 p.m.—Temperature 102.6°, pulse 132, respirations 60.
6 p.m.—Temperature 103.4°, pulse 160, respirations 56.
10 p.m.—Temperature 105°, pulse 138, respirations 40.
11 p.m.—Temperature 104.4°, pulse 150, respirations 40.

29th January, 1928—

1 a.m.—Temperature 106°, pulse 152, respirations 36.

Late in his illness he developed projectile vomiting and Cheyne Stokes breathing. The back appeared to be arched with the onset of convulsions. The cyanosis was profound and the limbs were stiff before death, which occurred at 2.45 a.m. on 29th January, 1928. (For temperature chart, see Appendix 8.)

Case 19—*W.R.—male, aged four years and two months.*—There was no history of diphtheria. He was inoculated on 20th January, 1928, and again between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was at 9.45 p.m. with vomiting and diarrhoea. His temperature at 6 a.m. was 103°. About 8.15 a.m. he had a convulsion and, at about 9 a.m., was seen by Dr. McKeon, who, after staying with him some time, and administering 4,000 units of antitoxin subcutaneously, had him admitted to St. Vincent's Hospital at 11 a.m.

He was then deeply cyanosed, unconscious and pulseless with shallow breathing. Temperature 102.8°. He had occasional convulsions in which the hands were clenched, arms and legs extended and the head slightly retracted. These spasms followed a little whine which could just be heard. The jaw stiffened and the mouth remained slightly open. The child vomited just before death, which occurred at 11.45 a.m. on 28th January, 1928.

Case 20—*M.R.—male, aged one year and eleven months.*—There was no history of diphtheria. He was inoculated on 20th January, 1928, and again between 4 and 5 p.m. on 27th January, 1928. The onset of symptoms was with vomiting and diarrhoea at 11 p.m. on 27th January, 1928. He was seen by Dr. McKeon at 9 a.m., and admitted to the Bundaberg General Hospital at 12.45 p.m. on 28th January, 1928, temperature 104°, pulse 160, respirations 60. His colour was pale and flushed by turns and his pupils were dilated. An enema gave a green slimy offensive result. Soon after admission he was convulsing, cyanosed, and later vomited. At 2 p.m.—temperature 104.2°, pulse 152, respirations 60. He died at 4.30 p.m. on 28th January, 1928.

Case 21—*E.D.—male, aged seven years and one month.*—There was no history of diphtheria. He was inoculated on 20th January, 1928, and 27th January, 1928. Abdominal pain and diarrhoea occurred at between 6 and 7 a.m. on 28th January, 1928. He then felt alright and went fishing until Dr. Thomson arrived at 10.30 a.m. and sent him to the Bundaberg General Hospital, where he was admitted at 11.30 a.m.—temperature 100.5°, pulse 118, respirations 38. An enema gave a green slimy offensive result. He was somewhat flushed and had a definite area of inflammation at the site of inoculation, which was tender. His temperature and respirations were down in the evening but his pulse rate remained high. The next day his temperature ranged from 98.2° to 99.2°, pulse from 100 to 120 and respirations from 20 to 28. He vomited a quantity of watery fluid at 6 a.m. and again later in the day.

His blood was cultured on 31st January, 1928, with negative result. He still had a raised pulse rate though his temperature was practically normal. By 1st February, 1928, he was much better. On 6th February, 1928, the arm became more inflamed. There had been a slightly increased temperature since the 3rd. On 8th February, 1928, a deep area of inflammation appeared on the buttock—this broke down and discharged on the following day. On 13th February, 1928, both the arm and buttock were widely incised by Dr. Hains and cultures were made from both by the Commission. A staphylococcus was found in each case. (Appendix 22.) (For temperature chart, see Appendix 8.)

TABLE 2.

SUMMARY OF THE ILLNESSES OF CHILDREN WHO RECEIVED THEIR SECOND INOCULATION ON 27TH JANUARY, 1928.

No.	Age.	Sex.	Time of Onset.	Symptoms.	Result.
14	Three years and two months	M.	5 hours ..	Early vomiting and diarrhoea, unconsciousness, rapid breathing, cyanosis, pyrexia, dilated pupils. Later convulsions. Late vomiting	Died 19 hours
15	Five years and nine months	M.	5 hours ..	Early vomiting and diarrhoea. Unconsciousness, cyanosis, pyrexia, rapid breathing, dilated pupils. Convulsions, restlessness. vomiting (late)	Died 24 hours
16	Three years and six months	F.	7 hours ..	Dry retching, later vomited. Conscious until 9 a.m., 29th January, 1928. Pyrexia dilated pupils, cyanosis, rash, convulsions, late projectile vomiting	Died 25 hours
17	One year and three months	F.	8 hours ..	Pyrexia, vomiting, rapid pulse and respirations, local inflammation, later abscess at site of inoculation	Recovered
18	Five years and six months	M.	5 hours ..	Vomiting and diarrhoea. Pyrexia, late unconsciousness, blotchy cyanosis and dilated pupils. Twitching of face and hands. Late projectile vomiting and Cheyne Stokes breathing. Limbs stiff before death	Died 34 hours
19	Four years and two months	M.	5¼ hours ..	Vomiting, diarrhoea, pyrexia, convulsions, cyanosis, coma, shallow rapid breathing, vomiting (late)	Died 18 hours
20	One year and eleven months	M.	5½ hours ..	Vomiting and diarrhoea. Pyrexia, dilated pupils, convulsions and cyanosis (late), also vomiting	Died 24 hours
21	Seven years and six months	M.	15 hours	Diarrhoea, pyrexia, local reaction leading to abscess at site of inoculation, abscess on buttock	Recovered

In this group there were eight children—six males and two females. Their average age was four years and one month. Two only recovered and, unlike the first group, there were no cases without symptoms. The higher morbidity and mortality is seen to be correlated with increase in dosage of the injected material. Despite the lower average age of the children comprising this group, these facts favour the view that the injection itself was responsible for the deaths and not some unrelated cause.

The differences between the two groups are not such as are met with in the comparison of the effect of a dose of toxin in the region of the M.L.D., with one twice as large, and may be held to suggest the intervention of a living organism. The figures are, however, too small to exclude error due to individual variation in susceptibility.

ANALYSIS OF SYMPTOMS AND DISCUSSION OF THEIR SIGNIFICANCE.

In the majority of fatal cases as well as in those which were severely ill, the symptoms and signs are so closely similar that the description of a single case, with one or two exceptions only, would adequately describe all of them.

Onset of Symptoms and Course of Illness.—The onset of symptoms was between 9.15 p.m. on 27th January, 1928, and 1 a.m. on 28th January, 1928—five to eight hours after the inoculations were given. In the severe cases (Nos. 1, 3, 4, 5, 6, 7, 11, 12, 13, 14, 15, 16, 18, 19, and 20), fifteen in all, there was vomiting at the onset which was followed soon after by several loose foul-smelling actions of the bowels. Diarrhoea only occurred during the early phase of the disease, and by the time the children reached hospital had ceased. Enemas and bowel wash-outs then yielded green slimy motions, with mucus in some, but no blood. In case 16, there was no diarrhoea. The vomiting also ceased early, but vomiting of bile stained, and later still of brown material (? stained with altered blood, due to continuous vomiting) was a late almost terminal event in Nos. 3, 4, 5, 14, 15, 16, 18, 19 and 20. This last vomiting was most probably of central origin and due to bulbar anoxaemia.

In all cases where the temperature was observed, there was pyrexia, which appeared to reach its highest level some hours after the onset of symptoms and which declined fairly rapidly in the case of children who recovered. The pulse rate was extremely rapid and out of proportion to the temperature, as also was the respiration rate. In cases which recovered, it took some days longer for the pulse to come back to normal than for the subsidence of the temperature. The

respirations appear to have been shallow and exceedingly rapid, and the picture in most of the severely affected children was, at the height of the disease, one of profound circulatory collapse and suggested the action of some powerful toxin on the vasomotor centre or directly on the peripheral circulation. In from eight to twelve hours from the onset of symptoms the children who subsequently died became unconscious with equal widely dilated pupils.

The Cyanosis and Convulsions.—The last stages of the disease were in nearly all cases associated with deep blotchy cyanosis and convulsions. The latter may have been asphyxial in origin or due to the action of some potent toxin on the cells of the cerebral cortex. It is uncertain, however, whether the cyanosis was not in most cases the result of the convulsions. So far as can be gathered from the narrative, they were sometimes preceded, but generally accompanied, by the cyanosis, and as was to be expected, occurred with greater frequency in the younger children. Case 1 had cyanosis without any definite convulsions. In Case 3 it is uncertain whether the cyanosis preceeded or resulted from the convulsions. The child was pallid at 2 p.m. and convulsions and cyanosis appeared half-an-hour later. Case 6 was deeply cyanosed, but had no convulsions. He had, however, some twitching of the face and shoulders in the early morning of 28th January, 1928. Case 11 was pale and later cyanosed, but had no convulsions. Case 12 was not cyanosed and there were no convulsions. Case 15 was very cyanosed before the onset of convulsions. In Cases 18 and 19 the cyanosis accompanied the convulsions. In Case 20 the colour was alternately pale and flushed and cyanosis only appeared with the convulsions.

Nervous Signs and Symptoms.—The pupil reflex was absent for some time before death in some cases. In only one was there definite rigidity before death (Case 18). There was no trismus, no emprosthotonos or opisthotonos, although in Case 18 the back was arched with the onset of convulsions. There was no local rigidity near the site of the inoculation. There was no head retraction. No paralyses were observed and no squints except in the doubtful case of one of the S. children, and then only during the convulsions. No changes were recorded in the reflexes before the onset of convulsions. In most cases irritability of a " cerebral " type was a conspicuous feature. Some of the fatal cases developed irregular or Cheyne Stokes breathing before death. Most of these cases had retention of urine, which by the second day, in the cases with severe symptoms which recovered, had passed off.

Rashes.—In two of the acute cases there was a rash on the body (Case 13, which recovered, and Case 16, which died). In the latter there was a generalized rash on the body on admission at 11.30 a.m., and though she had had anti-diphtheritic serum before admission, the period was too short for the rash to have been due to serum sickness. In Case 13, serum was given at 10.30 a.m. on the 28th and the rash was out by noon. This is unlikely to have been a serum rash, as they do not commonly appear on the first day. It was very irritable and general, extending to the feet and hands. When the child was seen by the Commission on 13th February, 1928, there was definite desquamation on her feet and hands. The rash in Case 16 was probably of septic origin and unrelated to the administration of serum, and the same may be true of that in Case 13, though the marked irritability after 48 hours and the spread on the extremities with later desquamation are equally favorable to the view that it was an unusually early serum rash.

Dr. Hains drew our attention to the condition of the tongue, which in most of these cases had a bright red area down its centre.

One case (No. 1) is said to have had its onset " rattling his cot with the shivers." A rigor in a child of two and a half years must be a rare phenomenon, onset with convulsions being more common at that age.

Symptoms in Survivors.—The children who survived the injection on the 27th January all present one important feature in common. All had abscesses at the site of the inoculation, from which, in five cases, staphylococci were isolated in pure culture. (Appendix 22).

Case 21, E.D., also had a marked local reaction and only very slight general symptoms, apart from a brief attack of abdominal pain and diarrhoea twenty hours after the inoculation, followed by pyrexia with two attacks of vomiting on the second day. The pulse rate was disproportionately high in this case also. He later developed a deep abscess in the buttock, from which a staphylococcus was isolated (different from that isolated from the abscess in his arm at the site of inoculation (Appendix 22). He was said to have had no antitoxin injected into the buttock, and this statement is supported by the examination of his serum taken on 15th February, 1928, for its content of diphtheria antitoxin, which was much lower than that in the children who were treated with antitoxin (Appendix 28).

In this case (E.D.), that of B.P. (Case 13), and that of J.S. (Case 7), blood cultures were made on 31st January, 1928 and 30th January, 1928, and examined by Dr. Richards. They were only incubated four days and were then discarded as they had grown nothing. J.S. and E.D. were almost afebrile, and B.P. had only slight pyrexia at the time at which the cultures were taken, so that had bacteriaemia been present earlier, it might have been missed at this time.

Case 10 (V.T.C.) had only a slight headache and a temperature of 100° twenty hours after the injection and later a local abscess.

C.W. and N.W. (Cases 8 and 9) had no immediate symptoms, but only a local abscess appearing later. No observations on the pulse rates are available in these children.

Case 7 (J.S.), the oldest child in the inoculated group, had moderately severe symptoms with vomiting and diarrhoea at the onset. There was pyrexia with disproportionately increased pulse rate, restlessness, retention of urine, and stupor. Like the other children who recovered, he had an early local reaction at the site of inoculation, where he later developed an abscess.

Case 6 (W.S.) was the most severely ill child who recovered. He had early vomiting and diarrhoea, high pyrexia and unconsciousness. There was twitching of face and shoulders, restlessness and retention of urine. He, likewise, developed a pleurisy on the second day and later an abscess at the site of inoculation.

Finally, Case 2 (F.B.) developed no symptoms at all apart from the late abscess.

The treatment of these cases is briefly described in Appendix 8.

OUTSTANDING DEFECTS IN THE CLINICAL STUDY OF THESE CASES.

In pointing out, briefly, some of the defects in the clinical study of these cases, having regard to its value in this investigation, it must not be thought that we are reflecting unfavourably on the medical practitioners at Bundaberg, who in the absence of specialist aid did nearly all that was possible in the short time available.

Blood cultures from two or three of the less seriously ill children taken on the morning of 28th January, when the pyrexia was at its height, would have been of great value, so also would leucocyte counts and a few blood films. Broad, general observations on the nervous system, such as the presence or absence of head retraction and of Kernig's sign would have been useful also. Lumbar puncture might with advantage have been done in two or three of the cases. The description of the order and appearance of symptoms is in some cases vague and the exact description of the symptoms themselves leaves much to be desired.

It was not possible to obtain microscopical and bacteriological examinations of the urine and stools. Somewhat unexpectedly no albuminuria was recorded in any case and we do not know whether or not pus cells, red blood corpuscles or micro-organisms were present in the urine.

It must be borne in mind, however, that confronted with the problem of treating a number of mortally sick children, it would have been very difficult to meet all these requirements.

THE PATHOLOGICAL INVESTIGATIONS.

The Autopsies at Bundaberg.—The police authorities, on learning that a number of deaths in children were occurring at Bundaberg in circumstances in which the medical attendants were not prepared to sign death certificates, ordered that Dr. Schmidt, the Government Pathologist, should perform autopsies on the bodies of all the victims of the tragedy. This instruction was not completely carried out. Nine autopsies in all were performed. At 3.15 p.m. on 28th January, 1928, Dr. Schmidt examined the body of one child (W.F.), and in the evening, between 7 p.m. and 10 p.m. (the exact order and times not being specially noted) the bodies of six others. The Commissioner of Health at this stage told the Commissioner of Police, in answer to inquiry, that it would be unnecessary to do more if Dr. Schmidt was prepared to give certificates of death in the remaining cases. Dr. Schmidt, however, decided to perform one more, which he did at 11.30 a.m. on 29th January, 1928, and in this case retained specimens of most of the tissues. One body (M.Br.) was held over to permit Dr. Richards to be present, and the examination on this case began at 10 p.m. on 30th January, 1928, i.e., 52½ hours after death.

No post-mortem examination was made in the cases of Monica S., Maisie S., and W.R.

Organs or portions of organs were retained from only four of the bodies, W.F., J.P., T.R., and M.Br. In the case of M.Br., specimens were retained for microscopic examination only. Of the others in two (W.F. and T.R.) the more important organs were preserved, though only in one (T.R.) d d these include the whole intestinal tract and brain. In the third (J.P.), two organs, a portion of another and a piece of clot, are all that were kept. Cultures and smears were taken in one case (M.Br.).

The tissues from W.F., J.P., and T.R. were preserved in methylated or rectified spirit and covered with gauze wet with the fluid on account of the insufficient quantity available.

On being taken over by the Commission on 14th February, 1928, the specimens were suitably incised and transferred to a large volume of 5 per cent. formalin in hermetically sealed tins for transport.

Résumé of Pathological Findings by Drs. Schmidt and Richards.—As the Commission had no opportunity of being present at an autopsy, they have had to rely for much that is of importance chiefly on the evidence of the operator, Dr. Schmidt, and of Dr. Richards, who assisted at the examination of one of the cases. Drs. McKeon and Hains were present at several autopsies, and have also given evidence on their observations.

There is a remarkable uniformity in the phenomena observed in the cases which have been examined pathologically in detail.

The early, apparently almost instantaneous, onset of rigor noted by a number of observers had completely or almost completely disappeared by the time of autopsy. In each case there was considerable bluish discolouration of general distribution on the surface of the body and limbs and less noticeable on the face, but with darker patches here and there, not hypostatic and with no special prominence or pigmentary demarcation of the veins. Dr. Schmidt did not think there were any haemorrhages into the skin. The glands in the neck and groin were not enlarged. The serous cavities contained no excess of fluid nor was this abnormal in character, and no hæmorrhages, exudate or other abnormal appearances were observed on the serous surfaces. The hearts were firmly contracted, with no observable abnormality in musculature, valves, chambers or endothelium. The contents were red clots and not fluid blood. Clots apparently of antemortem character are described as being a constant phenomenon in both venae cavae. The lungs were said to be a peculiar pink colour but free from congestion, hæmorrhages, oedema or consolidation. The mediastinal glands did not attract notice at the autopsy and the thymus presented no unusual features.

The stomach and intestines gave no indication of the presence of a lesion recognizable on examination from the peritoneal surface. Indeed the evidence is emphatic that they appeared to be anaemic. No localized contraction or dilatations were observed except in the case held over. The alimnitary tract appeared empty and collapsed. No congestion or lesion of the mucosa nor blood-stained mucus in the bowels was seen, though it must be pointed out that the examination of the interior of the bowel seems to have been very limited indeed. The liver called for no special comment. The spleen was very congested, but not enlarged. The kidneys are reported by Dr. Schmidt as normal except in one case (J.P.) where a dark-red area at the lower pole, thought to resemble an infarct was seen. Nothing abnormal was observed about the pancreas, great vessels, or gall bladder, and the lymphatic glands did not attract attention. The suprarenals were not found at any of the autopsies. The examination of the brain and its membranes revealed the presence of meningeal and superficial cerebral congestion and possibly pin-point hæmorrhages into the substance of the brain. The site of inoculation was not specially observed.

The above is the substance of Dr. Schmidt's observations. It was supplemented in some of the earlier cases by those of Drs. McKeon and Hains.

Dr. McKeon particularly noted the meningeal congestion and confirmed Dr. Schmidt's observation of the possible presence of an infarct in one kidney, and the excessive congestion of the spleens, which in the cases he saw at autopsy (W.F., T.R., and M.Br.), he thought were enlarged. He could express no opinion on the nature of the clots.

Dr. Hains was particularly struck by the anaemic appearance of the gastro-intestinal tract, and the injection of the surface of the brain, and observed on section general congestion in the kidney of W.F.

Dr. Richards has put his observations at the autopsy on M.Br. on record in a report to the Director of the Division of Tropical Hygiene (Appendix 10). They differ in several points from those of the observers who were present at earlier autopsies and from his own evidence given before the Commission. He was inclined to the opinion that the darker patches of the blotchiness observed in the skin were due to haemorrhage. He did not observe clots in the heart or great veins and fluid blood is mentioned as present in the heart. The spleen in his judgment was not enlarged or apparently engorged. No noticeable congestion was present in the meninges or brain, which, however, showed much post-mortem deterioration. Of the alimentary tract, the stomach was the only part laid open, and the mucous membrane was without congestion or lesion. There was some distension of the intestines with gas and a comparatively greater distension of the stomach.

Dr. Richards also reports on microscopic sections taken from the heart, lung, liver, spleen, pancreas, meninges, and brain, and on cultures from meningeal and pericardial fluid, liver, spleen and heart blood. Smears of liver and splenic tissue and films from the heart blood were also obtained. The suprarenal was not found in the specimens retained.

His microscopic examination of the sectioned material revealed no pathological changes in liver, heart muscle, spleen, lungs or brain. He describes changes considered degenerative in the pancreas, and toxic changes in the convoluted tubules of the kidney.

The cultures from the pericardial fluid were sterile, those from the heart blood, brain, liver and spleen showed putrefactive organisms.

The smears from the liver and spleen contained a number of organisms, amongst which short chains of cocci, possibly streptococci, were seen.

Additional Findings by the Commission.—These findings are the result of the examination of material obtained, as already described, from four of the children who died (Appendix 11). In three cases the autopsy was begun within eight and a half hours of death, in the fourth 52½ hours had elapsed. The advantage of the earlier autopsies was lost by the subsequent treatment of the specimens.

A detailed examination confirms in a measure the previous observation, but in some points leads to quite different conclusions. Dr. Schmidt's statements concerning the condition of the heart, stomach, pancreas, great vessels and gall bladder are substantiated. The lungs, however, show much congestion, especially of the larger vessels, and microscopically congestion in variable degree is present in the alveolar walls, with in some cases œdema and in all some alveolar dilatation and slight bronchial catarrh. The bronchial glands are definitely swollen and markedly engorged. Vascular congestion is also seen in the heart and thymus. Comments on the condition of the surfaces of organs are not of much value, but as far as the examination goes the absence of hæmorrhages, exudate or other lesion is confirmed. The oesophagus at its lower end and the stomach and duodenum show no gross recognizable change. Prominence of the lymphoid tissue increases towards the ileocaecal junction, where the mucous membrane generally is somewhat swollen and beyond that point the lymphoid tissue continues to show up conspicuously. Microscopically, there is no ulceration, hæmorrhage or other lesion to be seen, and this is confirmed microscopically, except that there is much disappearance of exposed epithelium, probably chiefly post-mortem, which limits the value of the observations. The anaemic appearance recorded at autopsy does not apply to the condition of the submucosa, which is quite definitely, though not excessively, congested. All the abdominal glands show moderate swelling and microscopically at least some vascular engorgement. The appearance of the lower margin of the liver suggests some volume increase. Section showed no recognizable change beyond some dilatation of hepatic veins.

The spleen in two cases is larger than usual and in the other case much smaller. Nevertheless, all are congested and all show evident swelling and pallor of the lymphoid tissue. The kidneys show engorgment of the larger blood vessels, but no recognizable focal lesion. If the kidney of J.P. retained is the one in which the infarct was suspected, no evidence now remains of its presence. The suprarenals were found in two cases (both organs in W.F. and one in T.R.) and present no sign of any pathological condition macroscopically or microscopically. The brain shows much superficial congestion and in one case (M.Br.) microscopic hæmorrhages near the surface, but no other abnormality is evident. Post-mortem changes in the pancreas are too advanced to allow of any useful histological examination.

Due allowance being made for post-mortem change, there would appear to be some cloudy swelling of the liver cells and of the epithelium of the convoluted tubules of the kidneys. Apart from this the liver exhibits a curiously uniform and the kidney a somewhat patchy congestion. No focal lesion or fatty change is found in either, but a certain leucocytic excess is noticeable in certain areas in the portal tracts. The most definite microscopic picture is that of the lymphoid tissue, which is everywhere swollen and somewhat hyperplastic.

The phenomena shown in the centres of lymph follicles whether in glands, intestinal mucosa or malpighian bodies of the spleen are similar throughout, fragmenting nuclei and granular degenerating material being phagocyted by proliferated endothelial cells. Bizarre nuclear degenerative forms are common and are seen not only here but variably in the splenic pulp or in the lymph cords and sinuses of lymphatic glands. In addition, in the glands at the root of the lungs (which are intensely engorged) and of the porta hepatis in two cases, the afferent lymph sinuses are notably dilated and contain large numbers of polymorph leucocytes, while proliferation and desquamation and phagocytic activity of endothelial cells in the sinuses complete a striking picture. The thymus does not share in this change and gives the impression only of a very moderate degree of œdematous swelling. The tissues from the site of inoculation, the bone marrow and the spinal cord were not included in the material retained from the autopsies.

Bacteria are present in the clot from the inferior vena cava of J.P. and in many organs. Many of these are coarse gram-positive bacilli, probably putrefactive. They are found in varying numbers on the peritoneal surfaces, in the clot, and in all the organs of M.Br. They are scanty or have not been found in the tissues from the other cases. In the clot there are also abundant staphylococci chiefly distributed in clumps or colonies round the margin of the fibrinous portion. There are also streptococci and other bacilli in small numbers. In most of the organs

diplococcal and single coccal forms, apparently staphylococci, are present in small numbers, but never in such numbers as to leave the mind free from doubt of their bacterial nature. Attempts to obtain unequivocal evidence of the distribution of staphylococci in the tissues must be regarded as unsuccessful.

Defects of Pathological Procedure.—While the provision of death certificates following autopsy by a Government Pathologist may satisfy purely administrative requirements, from the point of view of a Commission endeavouring to establish a cause for the fatalities at Bundaberg, the performance of the autopsies by a practitioner without special training leaves much to be desired. His consciousness of insufficient special knowledge, equipment and facilities for carrying out a peculiarly difficult investigation led Dr. Schmidt to appeal for expert assistance. The early presence of expert assistance at Bundaberg might have made the present inquiry unnecessary.

The outstanding defects are, in addition to the fact that autopsies were not performed on every case, the failure to retain samples of all tissues from every case done, the absence of examination of or retention of specimens from the site of inoculation, the lack of any proper examination of the intestinal tract, the absence of any observations on the suprarenal glands, the omission of any bacteriological examinations, and the unfortunate method of preservation of such specimens as were retained.

There was no facility at the morgue for the storage of bodies at a temperature below that prevailing at Bundaberg. One autopsy was done during the hours of daylight, and six at night in from two and a half to three hours, a quite inadequate period for a thorough investigation. Organs or specimens of organs (and those incomplete) were taken from only four of nine bodies. No measurements of body length or weight or of the weight of the organs were made. The sites of injection of the toxin-antitoxin were not specially examined and no tissue, culture or smear was taken from them. No specimens of the axillary lymph glands were retained though it appears that those in the neck and groin were examined. Lymph glandular conditions elsewhere were not observed. The nature of the skin discolouration was not determined nor the presence or absence of skin lesions noted. The suprarenals though searched for diligently in the case of M.Br. were not seen at autopsy. The throat and œsophagus were not examined. The alimentary tract (particularly the intestinal portion) was not completely laid open in any instance and the intestines were retained from only one of the cases. No precaution to preserve stomach contents or examine bowel contents was taken. The bladder condition and contents were not observed or specially examined. The spinal cord and bone marrow were not examined or retained for examination. Facilities for laboratory investigations are not available at Bundaberg, and except in the case reserved (and by that fact the least likely to yield information of any value) no smears or cultures were taken from organs or body fluids.

No effort was made to preserve suitable samples of the various tissues in either routine fixatives or fluids appropriate to special investigation. The organs preserved were not incised to ensure access of the fixative used. The amount of fluid applied was quite inadequate and resulted in the exposure of organs to the air, while the use of methylated or rectified spirit for the preservation in bulk of organs (extensively smeared with blood) was unfortunate. Moreover, the fluid was never renewed.

No notes of the conditions observable only at the time of autopsy were made and there is in consequence conflict and uncertainty of observation as well as of opinion amongst those who were present.

IS THERE ANY POSSIBILITY THAT THE DEATHS WHICH FOLLOWED THE INOCULATIONS ON THE 27TH JANUARY WERE DUE NOT TO THE INJECTIONS BUT TO SOME OTHER CAUSE ?

Taking into consideration the nature of the symptoms presented by the affected children, we had to explore the possibility of (*a*) food poisoning, (*b*) acute gastro-intestinal infection, (*c*) heavy metal poisoning, (*d*) other acute disease unrelated to the injection. In collecting evidence the Commission only publicly heard the male parents, though privately a number of the mothers were interviewed. It was obvious that the latter would provide more detailed information. We, therefore, addressed to the mothers a questionnaire which they very kindly filled in for us. The information derived from this source is included here. We have sought some common factor in the history of the children on 27th January which might afford a clue.

School.—Some of the children were of school age and were pupils at schools situated in different parts of the city. As the school holidays had not ended, the place of attendance is not significant.

Location of Homes.—The attached map (Appendix 1) shows the distribution of the homes from which the children came. It will be seen that these are scattered. In conversation with parents we were not able to ascertain that the children who were inoculated on the 27th of January had recently been congregated at any picnic or entertainment or at any place of meeting other than the Municipal Chambers at which the injections were given.

Inquiries were also directed towards ascertaining to what extent parents and children were acquainted with each other, and no indication was found of any association which covered all the children involved.

Food and Drink on 27th January.—Particular attention was directed to the food and drink taken on 27th January. In no case was food, drink or sweets partaken of at the Council Chambers. Except in their own homes, the only children who partook of any food or drink, sweets or fruit on the afternoon of 27th January, were the following :—E.D. (Case 21) had two ice-creams during the afternoon, T.R. (Case 18) and W.R. (Case 19) had ice-cream and cake, and M.R. (Case 20) had some cake. E.B. (Case 15) had a lemon squash and cake and some apples and plums, and his younger brother K.B. (Case 14) also had some fruit in Bundaberg. N.W. and C.W. (Cases 8 and 9) had malted milk at a cafe in the city.

How the Children went to be Inoculated and went to their Homes—V.T.C. and G.M.C. (Cases 10 and 11) left home with their mother at 3 p.m., were inoculated immediately at the Council Chambers, left with their mother and went straight home. N.W. and C.W. (Cases 8 and 9) left their home about 3.45, had to wait about twenty minutes for their injection and afterwards left with their mother and had malted milk at a cafe and then went straight home.

E.D. (Case 21) was taken by his mother. They left home at 12.30 p.m. and visited numerous shops before going to the Council Chambers. He was inoculated at once on arrival and left with Mrs. Br. and his mother, going straight home.

W.F. (Case 1), aged two years and six months was taken by his mother, leaving home at 3.50 p.m. They had to wait about ten minutes at the Council Chambers and left to go straight home with Mrs. C.O.B. and her children (K.B. and E.B.).

J.P. and B.P. (Cases 12 and 13) left home at 3.50 p.m. They had to wait about twenty minutes at the Council Chambers and went straight home afterwards.

T.R., W.R., and M.R. (Cases 18, 19 and 20) lefthome at 2 p.m. and visited a confectioner. They had to wait about fifteen minutes for the inoculations, but went straight home afterwards.

K.B. and E.B. (Cases 14 and 15) left home at 2.30 p.m. with their parents. They visited a hairdresser, but were injected immediately upon arrival at the Municipal Chambers. They went straight home afterwards.

M.Br. (Case 16) was taken by her mother, leaving home at 2.30 p.m. They visited a draper's shop before going to the Municipal Chambers, had to wait about fifteen minutes and left with Mrs. D. and E.D. to go straight home.

F.B. and G.B. (Cases 2 and 3) had to wait thirty minutes at the Council Chambers before inoculation, but went straight home with their mother afterwards.

The four S. children (Cases 3, 4, 5, and 6) were taken by their mother. They had to wait fifteen minutes at the Council Chambers, but went straight home afterwards.

B.D. (Case 17) was taken by her mother. They had to wait fifteen minutes at the Council Chambers, but went straight home afterwards.

These facts do not allow of the supposition that on the day of the injections these children all partook of any common article of food or drink. Apart from the exceptions noted above, all the food consumed was prepared and eaten in their various and widely-separated homes.

The only experience they shared in common was their presence in the Engineer's Room at the Municipal Chambers between 4 and 5 p.m. and the injections which they received there.

Despite the unlikelihood of a common "food poisoning" or gastro-intestinal infection, we considered it desirable to examine the sera of those children who survived to ascertain whether in any of them there was evidence of antibody production to any member of the "Salmonella" group.

This was done for us by Dr. F. M. Burnet, of the Hall Institute, who at the same time tested the sera for agglutinins against the organism which was found in abscesses at the site of inoculation in every case in which it was sought.

Agglutinins in the Sera of the Children who Survived.—Sera from eight of the surviving children—J.S., W.S., C.W., V.T.S., B.D., E.D., B.P., and F.B.—were examined for their content of agglutinins against various Salmonella strains. The cultures used were from the National Collection of Type Cultures, London, but in the absence of homologous sera their agglutinability and their present state as to specific or non-specific phase could not be determined. In no case was any agglutination evident at dilution of 1 : 20 or higher. The following Salmonella strains

were used against all sera—B. paratyphosus B., B. enteritidis Gaertner, Salmonella types, Stanley, Reading and Derby, and B. paratyphosus C. (Hirschfeld). There is, therefore, no evidence of the presence of Salmonella agglutinins, and, moreover, the clinical evidence is unfavorable to the possibility under discussion.

At the same time the sera were tested with an agglutinating emulsion of the Bundaberg staphylococcus and four sera from children in Melbourne were used as controls. Agglutination was evident with all sera to the titres shown—

J.S.	80 + +	.. Controls.	
W.S.	40 + + +	.. G. ..	40 + +
C.W.	40 + + +	.. L. ..	40 + +
V.T.C.	40 + + +	.. H. ..	40 + +
B.D.	40 + + +	.. M. ..	40 + +
E.D.	40 + + +		
B.P.	40 + +		
F.B.	80 + +		

There is, therefore, only a very doubtful slight increase in the amount of staphylococcus agglutinin beyond the normal in children.

Discussion of Symptomatology and Pathological Findings.—The diarrhoea occurred soon after the onset of symptoms and by the time the children were admitted to hospital had ceased. Vomiting, it is true, continued, but the late vomiting seems to be clearly due to other causes. No blood was noted in the stools though mucus was present in some, and no bacteriological or microscopical examinations were made. The clinical picture only very superficially resembles that due to food poisoning or acute gastro-enteritis.

Furthermore, the pathological evidence is distinctly unfavorable to these possibilities. The serous surface of the small and large intestines was in all cases pale and not congested, though proper immediate examination of the mucosa was not made in any of the nine autopsies.

The post-mortem findings in the only case in which they have been fully investigated are also opposed to this hypothesis. Specimens of the whole intestinal tract were only available to the Commission in one case (W.R.), autopsied eight to nine hours after death. The value of this material was greatly reduced by the post-mortem changes (desquamation of cells, &c.) which had occurred. There was general lymphatic hyperplasia in the ileum and cæcum and some simple hyperplastic enlargement of the adjoining lymph glands, but no congestion, œdema, cellular infiltration, necrosis or ulceration was evident in any portion of the mucosa.

Finally, no other cases of severe gastro-intestinal disorder occurred in Bundaberg and its environs at this time. The evidence of all the doctors in the town is unanimous on this point. The whole evidence is, therefore, overwhelmingly opposed to the possibility that the deaths at Bundaberg on 28th and 29th January, following the inoculation of toxin-antitoxin mixture were due either to food poisoning or to acute gastro-intestinal infection.

The absence of any common food supply also excludes the possibility of botulism and the clinical picture does not bear the faintest resemblance to this disease.

The possibility of heavy metal poisoning has been suggested by more than one authority as a possible cause of the Bundaberg deaths. Administration with food or water by accident or intent is excluded by the evidence detailed above and the possibility of any heavy metal gaining access to the interior of the bottle is eliminated by the fact that we were able to isolate living organisms from its remaining contents—which would certainly not have been possible had any heavy metal been present in significant concentration. The needles used for the injections were in excellent condition, little used, and free from any obvious oxidation, so that this extremely unlikely source is excluded.

Further, the pathological findings already discussed, particularly those in the liver, kidney, and large bowel, are incompatible with acute death from any such cause. We have, however, had an analysis made of the livers of W.F. and M.R. (Cases 1 and 20) by Mr. F. H. Holden, of the Hall Institute. The results of these analyses are given in Appendix 12. No significant amounts of heavy metals were found.

That the deaths were caused by some other acute disease unrelated to the injection, in the absence of any other similar cases of illness among children in the City of Bundaberg, is too remote a possibility for serious consideration. The mere gathering of these children together in one place in which only a few of them were present at the same time cannot be regarded as providing even the slightest ground for the belief that while at the Municipal Chambers some of them were infected with a mysterious and fatal disease.

Finally, as has been earlier pointed out, the heavier mortality among those children who received the second and larger injection, and the general though not absolute relationship of the severity of the effect to the age, and presumably to the body weight of children in the same families, and finally the onset of significantly similar symptoms at approximately the same time interval

after the injections, in most cases, afford strong evidence *that the injection of toxin-antitoxin mixture on the 27th January was the cause of the deaths and the severe illnesses which followed only in children who had received the injection on that date.*

DO THE SYMPTOMS WHICH FOLLOWED THE INJECTION IN THESE CHILDREN FAVOUR THE VIEW THAT THEY WERE CAUSED BY FREE DIPHTHERIA TOXIN IN THE BOTTLE OF TOXIN-ANTITOXIN MIXTURE?

Clinical experience of diphtheria would not enable us to answer this question definitely though Dr. Scholes of the Infectious Disease Hospital speaking from his very large experience thought it unlikely that the symptoms were due to this cause.

Résumé of previous accidents.—Previous accidents in the history of immunisation afford us the only satisfactory evidence in regard to the symptoms which may be expected to follow the administration of free diphtheria toxin. The most important of these for our purpose is that which occurred at Baden, Vienna, in September, 1923, when seven children died as the result of the injection of a mixture supposed to contain toxin and antitoxin for active immunisation against diphtheria. According to Grassberger the deaths in this case were due to some of the phials being filled in error with toxin instead of with toxin-antitoxin mixture—though Busson and Loewenstein, who were responsible for making the mixture, held that the phials became toxic by the dissociation of free toxin from the mixture which according to them was originally atoxic.

This accident took place at an infant's home where two cases of fatal diphtheria had occurred and necessitated active immunisation to limit the spread of the disease. All the infants were first Schick tested to ascertain how many of them were immune, and thirty-four susceptible children received a subcutaneous injection of 1.0 c.cm. of the supposed toxin-antitoxin mixture in the abdominal wall. After twelve hours some of the children became restless and within twenty-four hours swellings appeared at the site of inoculation which soon spread widely to the axilla and groin (infiltration). Despite the subcutaneous administration of 2,000 units of antitoxin at this stage, there were seven deaths and ten cases of severe reaction. The deaths occurred in infants aged 50, 34, 30 and 144 days, 2, 1 and 1 years respectively. All had been Schick positive. In all the diphtheritic infiltration of the abdominal wall was very extensive (large vesicles appearing in the skin) and in some cases this was followed by necrosis. Death occurred on the 7th, 4th, 5th, 9th, 12th, 17th and 41st days. In all the fatal cases the typical hæmorrhagic infiltration of the adrenal glands was found postmortem. The two which died on the 9th and 12th days had each an excessively large thymus and two others were described as "weakly," the first being a premature child.

The toxin-antitoxin mixture made by Loewenstein and Busson should have contained in 1 c.cm., 14–19 M.L.D. for guinea pigs of 250 grams, over-neutralized with antitoxin. The actual material used for injection into the infants and children at Baden estimated by its toxicity for guinea pigs contained, according to Grassberger, about 10 -M.L.D.'s of free toxin in 1.0 c.cm.

Another accident occurred at Dallas in Texas. In this case forty severe reactions in children followed the injections from one particular batch of toxin-antitoxin mixture. In mixing this batch the Danysz phenomenon was overlooked and a toxic mixture resulted. The symptoms observed were pyrexia, vomiting and pain at the site of injection and in all of them there was an extensive local reaction, the skin being intensely inflamed and the inflammation extending to the forearm, shoulder and hand and even across the chest in some cases. Large vesicles filled with clear fluid appeared, presumably like those observed in the Baden disaster. Five deaths occurred in from twelve to sixteen days after the injection, but no postmortem examinations were made.

Still another accident occurred in 1924 at Concord and Bridgewater, Massachusetts, following the injection of toxin-antitoxin after it had been frozen during the intensely cold weather and then thawed out for use. Material from the same batch had been used earlier without any ill-effects but after freezing and thawing it produced severe local reactions and constitutional disturbances. G. W. McCoy, M. J. Rosenau and W. H. Park commenting on this accident recommended that in future mixtures containing 1/10 L + dose of toxin per dose for injection should be used—a strength which, it may be noted, had already been in use in Australia since 1922 when the toxin-antitoxin mixture was first made by the Commonwealth Serum Laboratories under Dr. Penfold's direction. This accident was clearly shown by the later work of Glenny and Pope to have been the result of the localized concentration of phenol in the mixture during the process of freezing with consequent destruction of antitoxin.

Consideration of toxin-antitoxin mixture used at Bundaberg—No method is readily available of dissociating toxin from antitoxin mixture so as to obtain a quantitative yield of free toxin, and we were not, therefore, able by experiment to exclude the possibility that Batch 86 was a

more concentrated mixture of toxin and antitoxin than it purported to be (Appendix 13) We have, however, checked the process of mixing from the original notes of the laboratory and have ascertained beyond question that these had in no way been altered since they were first written.

A large number of independent witnesses speak for the fact that the mixture was evenly balanced. No ill effects followed injections from a number of different samples of this batch in a large series of children, not did any such effects follow the injections made by Dr. Thomson prior to 27th January. In addition to the tests originally made when the batch was issued, special tests were set up immediately at the Commonwealth Serum Laboratories and these were satisfactory. Finally our own experiments showed that the mixture was properly balanced (Appendix 16).

Possibility of deaths from free toxin.—Gross errors in dosage by Dr. Thomson being excluded by the volumetric considerations in regard to the quantity left in the bottle, detailed in Appendix 18, the first immunising dose of two minims given at Bundaberg on 27th January could have contained only 0.1 L + dose and the second dose of four minims rather less than 0.2 L + dose even if all the toxin present had been set free. There is furthermore a very striking difference between the deaths at Bundaberg and those at Baden. The affected children at Bundaberg were not weakly infants like those at Baden but strong healthy children and even presuming that the whole of the available toxin in Dr. Thomson's bottle had been dissociated from antitoxin it could not possibly have caused the very rapid fatalities which ensued at Bundaberg. Further, none of the survivors in the three months which have elapsed since the injections have developed any palatal palsies or late effects from diphtheria toxin.

Even if it were supposed that the mixture had been made up wrongly and contained much more toxin and antitoxin than it purported to do, the deaths could not be explained by the liberation of correspondingly greater amount of toxin as there were no local reactions in any of the children injected on the 27th, which were in any sense equivalent to those which would have been produced by the subcutaneous injection of a significant amount of un-neutralized diphtheria toxin.

It is, however, not clear from the clinical evidence that dissociation of quantities of diphtheria toxin too small to produce infiltration might not conceivably have played some part in increasing the activity of the agent which actually caused the deaths, or of diminishing the resistance of the affected children. That this possibility is very unlikely is indicated by our experiments in Appendix 29.

History of children in regard to diphtheria.—Among the children inoculated on the 27th January, one (Case 9), gave a history of diphtheria a year before, one (Case 8), a doubtful history and one (Case 14) had been a carrier. The two first had no symptoms following the injection and the last died. The mother of Case 1 had had diphtheria in November, 1927. Two other children of the same family as B.D. (Case 17) were in hospital with diphtheria at the time when B.D. was inoculated, and a brother of M.Br. (Case 16) had just come out of hospital where he had been treated for diphtheria.

We twice swabbed all the survivors of the injections on the 27th January without finding any carriers of virulent diphtheria bacilli among them.

Antitoxin in blood of survivors.—In view of the possibility that diphtheria toxin might have played an adjuvant rôle in causing the deaths, we considered it advisable to ascertain whether there were any notable difference in the amount of diphtheria antitoxin in the blood of the survivors which might account for differences in the severity of symptoms.

Since it was not expedient to request the parents to allow us to submit these children to the Schick test, we obtained the sera of eight of the nine survivors and tested them for their content of diphtheria antitoxin (Appendix 28). The sera from two of four children who had no symptoms following the injections were found to have a very high titre of antitoxin. A third had an extremly low titre and we were not able to get serum from the remaining child. The sera of three children who recovered after a severe illness and who had received 4,000 units of antitoxin intramuscularly on 28th January, 1928, were found to have a moderately high titre of antitoxin. Finally, the sera of two children who had a less severe illness and to whom no antitoxin had been administered, had a very low titre of antitoxin.

It seems likely that the children who had a high titre of antitoxin in their circulating blood of 15th February, 1928, would have been protected against diphtheria toxin on 27th January, but a third child who also had no symptoms could have had no such protection.

With regard to the remaining children, it is not possible to infer from these results whether they were Schick positive or Schick negative before 27th January. The effect of the administration of antitoxin is still evident in the relatively high titres given by those who received 4,000 units

in hospital and it is not unlikely that, had they not received this treatment, the antitoxin content of their sera would also have been low. These results are unfavourable to the view that free diphtheria toxin was responsible in whole or in part for the deaths at Bundaberg.

There is no specific evidence that diphtheria toxin is the cause of the pathological lesions found. It should be remembered that most of the systemic lesions described as characteristic of diphtheria have been observed in cases in which there was a much longer time for their development than there was in the present series. It is true that the most striking lesions present in the histological material from these cases are those of the lymphoid tissue, but they are not demonstrably specific and may occur in other diseases in which the incidence of the poisoning is prominently in that tissue, more especially in children. There was no hæmorrhage in or about the lymph glands. In the only cases in which autopsies have been performed following deaths presumably due to the direct inoculation of children with diphtheria toxin, hæmorrhagic infiltration into the suprarenal glands was constantly present. No such lesions were present in the suprarenals examined from two of the present cases. There is an absence of change in the musculature or interstitial tissue of the heart, of focal or central necrosis in the liver, of localized cellular accumulations in the kidneys or lungs, and of phagocytic activity in the vicinity of the Hassel's corpuscles in the thymus. Characteristic infiltration of the tissues at the site of inoculation would probably not have been missed had it been present.

Experimental studies on toxin-antitoxin dissociation. — We have not been able to demonstrate the smallest amount of free toxin (1/250 to 1/500 of an M.L.D.) in toxin-antitoxin in which we have grown the Bundaberg staphylococcus at various temperatures (Appendix 26). Free antitoxin is destroyed only very slowly by this organism which we isolated in pure culture from the remaining contents of Dr. Thomson's bottle of toxin-antitoxin mixture (Appendices 18 and 25). Even the growth of an organism capable of rapid proteolysis (B. proteus x 19) does not appear to be capable of dissociating toxin from antitoxin mixture (Appendix 21). The assumption that dissociation was produced by some organism other than the staphylococcus, which multiplied and then died out, is inherently improbable upon this ground alone. When, however, we take into account the ease with which organisms like streptococci and B. proteus are recovered after growing together with staphylococci in toxin-antitoxin mixture for a week or more (Appendix 21), the stability of diphtheria toxin in the presence of the Bundaberg staphylococcus (Appendix 25) and our inability to demonstrate any trace of toxin in Dr. Thomson's bottle (Appendix 18), the possibility of such an occurrence becomes very slight indeed.

Taking all these facts into consideration, we may dismiss the possibility that dissociation of toxin from toxin-antitoxin mixture played any part at all in causing the illnesses and deaths under consideration.

Was tetanus the cause of the Bundaberg fatalities?— The second possibliity that must be discussed here is the suggestion that tetanus toxin might have been produced in the bottle in sufficient amount to give rise to rapid death, assuming that the contents at some time had become contaminated by B. tetani. Regarding this suggestion in the light of the clinical findings, it must be admitted that there is no clear evidence in its support. It is true that most of the fatal cases had convulsions, but these only occurred in the younger children. In the others there were twitchings of the shoulder and face. Dr. Schmidt who saw three of the children during life regarded the convulsions as those peculiar to young children and infants. Dr. McKeon in two of the fatal cases observed flaccidity between the convulsions. Dr. Hains, who saw most of the children in the late stages, observed a certain degree of rigidity between the spasms in some of them, notably in T.R. (whose back was somewhat arched with the onset of the convulsions). He was, however, quite definite that the rigidity he observed was not a tetanic rigidity and that the convulsions were not tetanic. There was no suggestion of any local rigidity in the neighbourhood of the site of injection and in no case was there rigidity of the abdominal wall, or trismus and in only one (Case 18) were the limbs stiff before death.

Retention of urine and early unconsciousness observed in the fatal cases also form no part of the picture in tetanus, nor do the gastro-intestinal disturbances present in nearly all the Bundaberg cases.

The amount of tetanus toxin required to be generated in the bottle must necessarily have been very large, despite the extreme sensitiveness of man to this toxin, to permit of the time from injection to death being so short. If B. tetani had multiplied in the bottle to a sufficient extent to liberate this amount of toxin, it is inconceivable that in the surviving children, who subsequently developed abscesses at the site of injection, living tetanus bacilli should not also have flourished and given rise to chronic local tetanus or more probably to acute tetanus with its normal incubation period of some days.

The pathological findings, as might be expected, do not provide any evidence either for or against this hypothesis. The nervous system was not in suitable condition for examination by special staining methods to demonstrate changes in the dendrites, granules or nuclei, and in any case the changes which have been described in tetanus are not specific.

Neither degeneration of voluntary muscle nor gross hæmorrhages nor rupture were described in any of the Bundaberg autopsies.

Attempts to grow tetanus bacilli (Appendix 20) both in the presence of the Bundaberg staphylococcus and alone in rubber-capped bottles of toxin-antitoxin mixture, indicate that the conditions are unfavourable either for the growth of the organism or for the production of toxin.

We were unable to recover B. tetani from the bottle used by Dr. Thomson for his injections and the experiments in Appendix 20 show that these organisms when present even in small numbers are readily recoverable by culture in the media which we used for the investigation of the remains of the bottle of Batch 86 from which the injections at Bundaberg were made. On these various grounds, therefore, we can exclude the possibility that the deaths at Bundaberg resulted from tetanus.

Were the deaths at Bundaberg due to anaphylaxis or allergy?—The next question which arises is as to whether the symptoms could possibly have been anaphylactic or allergic in origin. It was evidently from this point of view that atropine and adrenaline were freely used in treatment.

Dr. Scholes who had had a large experience of the effect of serum injections, was of the opinion that the symptoms could not be explained in this way. There are some further general considerations which confirm this opinion.

In the first place it would be an extremely remarkable phenomenon if in a group of 21 children (eight of whom had previously been inoculated from the same bottle without any symptoms) twelve should die from allergy (using this term in the sense defined by Coca) and at least four others should exhibit severe symptoms. On the other hand, if anaphylaxis were presumed to be the cause and the first injection the sensitizing dose, the affected children should have been only in the group who received their second injections, and in any case the intervening period is too short for the acquirement of sensitiveness. Further, the nature of the material, its extremely small content of horse serum, the small size of the dose and the manner of the injection are unfavorable to this possibility, though in view of the case noted below they do not exclude it. The symptoms of the Bundaberg cases only very superficially resemble those produced in anaphylaxis or allergy. The time elapsing until the onset of symptoms is too long and the breathing which was shallow and rapid is unlike the type of respiratory distress which would be expected in at least some of the cases were they allergic or anaphylactic in origin. Finally the cases did not appear to respond to treatment with adrenaline.

In Dr. Derham's evidence he referred to an illness which may possibly have been anaphylactic in a girl of fourteen years of age who had had a previous injection of diphtheria antitoxin about three weeks before. About four hours after the injection of two minims of toxin-antitoxin mixture she developed pyrexia, a very rapid pulse rate, cyanosis and dyspnoea (resembling asthma, but not definitely expiratory). She responded well to an injection of adrenaline. Such an isolated case may have this explanation.

THE SIGNIFICANCE OF THE SYMPTOMS.

What then is the significance of the symptoms in the children who were inoculated on 27th January at Bundaberg? They were those of a profound and overwhelming toxaemia resulting either from the injection of preformed toxin or from the formation of toxin "in vivo," with or without septicaemia.

The toxin evidently had a profound effect on the cardio-vascular system whether exerted centrally or peripherally. The early vomiting and diarrhœa have no special significance. These symptoms are commonly met with as an early non-specific response to any toxin. The late picture was dominated by anoxaemia and cyanosis associated with the profound circulatory failure induced by the toxin. The convulsions may have been due either to a direct action of the poison on the central nervous system or to asphyxia.

For the further consideration of the cause of these symptoms it is necessary to take into account the results of our investigation of the contents of the fatal bottle (Appendix 18).

RESULTS OF THE INVESTIGATION OF THE REMAINING CONTENTS OF DR. THOMSON'S BOTTLE.—THE BUNDABERG STAPHYLOCOCCUS.

The remaining fluid was turbid, and, apart from a yeast which we failed to grow, the only organism which could be isolated from it was a staphylococcus which we have named the Bundaberg staphylococcus. This organism was found to be identical with staphylococci isolated in pure culture from abscesses at the site of injection of five of the surviving children (Appendix 22), In the remainder it was not sought for. Its pathogenicity for guinea pigs, mice and monkeys (macacus rhesus) is slight. In rabbits, intravenous injections in relatively small doses may give rise to death in 12 to 24 hours with convulsions and without gross lesions at autopsy. In animals surviving longer, gross suppurative kidney lesions are invariably found. Even for this species the pathogenicity of the organism is subject to wide variation.

Its morphology is in every way typical of the staphylococcus pyogenes group, i.e., minute Gram positive spherical cocci forming pairs, tetrads, short chains and clusters.

It grows readily at room temperature and somewhat better at 37° under both anaerobic and aerobic conditions. Its coloration on solid media is intermediate between the typical "aureus" and "albus." Twenty-four hours' growth on agar has a pale cream colour; longer incubation produces an orange tint which is more noticeable where the growth is heavy or is drawn up into a heap with a platinum loop. It forms a smooth and not viscid emulsion in physiological salt solution, and the emulsion runs rapidly through a moderately tight cotton-wool filter, differing in these respects from staphylococcus epidermidis albus.

On agar plates discrete surface colonies are flat but the centre is somewhat raised, and if the plates be left in the incubator there is some further thin peripheral growth which develops a finely dentate edge. The colonies are at first cream coloured but later the centres have an orange tint by transmitted light.

Plates occasionally showed more typically aureus variants often appearing as a sector change in a predominantly white colony. In platings from the original cultures in glucose broth after some weeks well-marked aureus colonies appeared as well as typical pale types. These colonies showed distinctly different serological behaviour referred to in Appendix 22. Deep colonies in agar plates are shaped like a double convex lens. Gelatin is liquefied, a small cup-shaped depression being visible at the lower end of the streak in eight days at room temperature.

In broth there is a smooth growth, but a deposit begins to form quite early. With a moderate inoculum there is a considerable turbidity and a little creamy deposit in 24 hours, both more marked after 48 hours. The deposit becomes buff coloured but does not develop the typical orange colour of the "aureus" deposit. Old cultures may show some scum on the surface, more particularly attached to the glass round the periphery.

On blood agar plates there is slow and only slight haemolysis, contrasting strongly with the typical haemolytic "aureus." After some days growth on human blood agar plates discrete circular areas of haemolysis appear in a zone adjacent to the regions of growth. These almost certainly represent the phenomenon described in 1927 by Muller as "haemophagie."

Glucose, lactose, saccharose and mannite are fermented with acid production in 24 hours. Salicin, raffinose and inulin are not fermented in nine days. Litmus milk becomes slightly acid in 24 hours, more strongly acid in two days and is clotted in four days.

A serum produced against the Bundaberg staphylococcus agglutinates most pyogenic aureus strains to its full titre.

The organism when grown at 37° C. for so short a time as seven to eight hours in broth, or in broth or toxin-antitoxin mixture to which human blood has been added, produces a filterable toxin, somewhat unstable in dilute solution, which reacts specifically in skin tests in man (Appendix 24). We have been able to show that there is a great deal of variability in the sensitiveness of different individuals to this toxin. In sensitive individuals good skin reactions may be obtained in dilutions of 1 in 2,000. In some cases better reactions are observed when human blood has been added to broth than when broth alone is used for culture.

It may be significant that the virulence of the organism for rabbits is enhanced by injecting the serum from blood from the heart of an animal killed by intravenous injection of the organism, after this blood has been incubated a few hours.

The substance which in such high dilutions give rise to skin reactions in man is non-toxic as judged by the results of injection in large doses into laboratory animals (guinea-pig, rabbit and monkey)—a point of interest in view of the low pathogenicity of the organism when tested by subcutaneous injection in these animals.

Pathogenicity of Bundaberg Staphylococcus in Toxin-Antitoxin Mixture.—We have also carried out suggestive experiments in which this organism has been grown in toxin-antitoxin mixture at Bundaberg temperatures. It is interesting that under these conditions a cellulitis is produced in guinea-pigs following the subcutaneous injection of 0·5 c.cm. of the mixture at about the sixth day. The power to produce this reaction is then lost but returns at about the ninth day. Since similar infiltrations can be obtained by the injection of an emulsion of the organisms in sufficient number together with 0·5 c.cm. of nutrient broth or of toxin-antitoxin mixture, this phenomenon is difficult to interpret. Its development appears to depend on the presence in sufficient numbers of both living and dead organisms rather than on the number of living organisms, but the disappearance and re-appearance of activity remains unexplained. Individual variations in the resistance of the experimental animal only exaggerate, but do not explain the effects produced.

Did the Deaths at Bundaberg result from the Activity of this Staphylococcus ?—Let us now consider in the light of these facts whether it is possible that the illnesses and deaths at Bundaberg resulted from the subcutaneous injection of toxin-antitoxin mixture containing this organism in considerable numbers. In Appendix 27, we have given reasons for thinking that on 27th January, in an injection of two minims there were either (*a*) at least 130,000, or (*b*) 5,000,000 living staphylococci. These estimates are extremely conservative. (*a*) is based on the assumption that Dr. Thomson was correct when he stated that the contents of the bottle were turbid on the morning of 28th January, but clear on 27th January, when he made the injections, and (*b*) is based on the probability that the bottle was contaminated on 24th January, as we think was almost certainly the case.

Discussion.—In considering the clinical and pathological data in relation to these facts, it must be borne in mind that, though some of the earlier bacteriologists injected staphylococci into their subcutaneous tissues with striking local effects, *there is not, within our knowledge, any previous experience of the results of the subcutaneous injection of large numbers of living staphylococci into young children.* The results of clinical experience of staphylococcal infections are not strictly analogous to the cases under discussion. In the sudden dissemination of staphylococci from a pre-existent focus which sometimes results in staphylococcal septicaemia and pyaemia, the process is taking place in the body of an individual who has presumably acquired some degree of immunity both local and general to the organism and its products. Such general staphylococcal infections are rarely fatal in less than 48 hours though a few in which death occurs at about this time have been described. They have an exceedingly high mortality, *vide* Osler's *Modern Medicine* 1925, Vol. 1., p. 547, where according to Jacob, the mortality for various infecting organisms is given as staphylococcal, 88 per cent., streptococcal 83 per cent., pneumococcal 52 per cent., and due to the colon bacillus 41 per cent.

We ourselves have seen several rapidly fatal staphylococcal septicaemias in which collapse and stupor or unconsciousness have been prominent features.

In the profound toxaemias produced in general staphylococcal and streptococcal infections, the pulse tends to be rapid out of proportion to the temperature. This sign was characteristic of the Bundaberg cases, but can only be regarded as the effect of toxin on the peripheral vascular system or on the bulbar centres. The cerebral symptoms would accord well with fulminating staphylococcal infection.

Instances are on record of rapid death in children following operative interference in osteomyelitis with entry of staphylococci into the surrounding normal tissues.

We are indebted to Dr. Paterson, Superintendent of the Hospital for Sick Children, Brisbane, for the following histories of acute staphylococcal osteomyelitis obtained from the Hospital records of the last three years which illustrate how extremely rapid the course of fatal cases of this kind may be :—

> C.P., male, aged two years. The child awoke this morning feverish and the right knee was seen to be swollen and extremely tender to touch. He commenced vomiting about mid-day and the bowels have since been rather loose. He was admitted at 9 p.m. At 10.5 p.m. the upper end of the right tibia was explored and thin pus found from which staphylococcus aureus was cultured. He died at 3.45 a.m. after an illness of less than 24 hours.
>
> E.N., male, aged eleven years and nine months. He had had slight pain in the right knee for two days. This morning he was convulsed and since has been delirious. An enema was returned with blood stained fluid and faeces. He was admitted at 11 p.m. and operated on two hours later. The lower end of the right femur was explored and a large amount of brown fluid pus obtained which yielded staphylococcus aureus on culture. He died at 6 a.m.

W.D., male, aged two years and ten months. His illness began with convulsions and vomiting last evening and continued this morning. He would not use his right arm. He was wrongly diagnosed as infantile paralysis and left in the ward ten hours before being seen by a Senior Surgeon and operated upon. The upper end of the right humerus was explored and thin pus found from which staphylococcus aureus was cultured. The child died shortly after operation.

Dr. Wanliss has examined for us the literature and the records of some hospitals for fulminating cases of staphylococcal infection. These cases are rare, but by no means unknown. A contributory cause to their rarity is that only a short time is available for investigation before death, and that at autopsy no gross lesions are found. A certain number of these obscure cases are met with in infants and children. The following cases in adults are worthy of particular notice :—

Cabello (*Med. Ibera*, 1925, p. 235—cited by Herrick in Nelson's loose-leaf Medicine, December, 1925), reports a case of septicaemia due to staphylococcus aureus in a young man, who died 48 hours after an operation for hernia. In this case it is noticeable that though staphylococci were isolated from the wound and the heart blood no other lesions except endarteritis of the femoral artery were found.

Vincenzi (*Deutsch. Medizin, Woch*, 1906, p. 1039) records a case of acute infection which was fatal within three days and which occurred in a healthy and strongly-built young man of 21. It originated in an acute lesion of the lower lip, which was mistaken for anthrax. Staphylococcus pyogenes aureus was recovered in pure culture from a deep puncture of the tissues underlying the lesion. Restlessness, extreme cyanosis, difficulty in breathing, delirium, pyrexia (104·5° F.) and later collapse were the main clinical features. The most notable feature of this case was that the organism unlike nearly all staphylococci recovered from acute fatal septicaemias in man (which, according to this author, have only slight or no virulence for laboratory animals) had a high degree of virulence. 0·1 c.cm. of broth culture (age not stated) injected subcutaneously, killed mice with oedema spreading from the site of injection in eight to twelve hours. The same dose killed guinea-pigs in two or three days with a similar haemorrhagic or serous infiltration (*vide* Appendix 26). Rabbits of 1.5 K. were killed in three days.

French (Guy's *Hospital Reports*, LXIV., p. 343), describes the case of a woman, aged 33, who died of acute staphylococcal infection on the third day without any lesions at autopsy other than cloudy swelling of organs and subpleural and subdural petechiae. She had apparently tried to open a whitlow in her thumb with a knitting needle. Her illness commenced with a rigor, on the next day she vomited and developed a high temperature, 105° F. Twenty-four hours from the onset she managed to walk to the hospital, but quickly became comatose, had slight diarrhoea and died the following day. Blood culture on the third day and, at autopsy, cultures of the spleen, heart blood and pericardium all yielded a pure staphylococcus aureus.

Let us consider what might happen following the introduction of large numbers of virulent staphylococci into the subcutaneous tissues. Variations in the effects produced in individuals would presumably depend either on the nature of their local resistance or on the degree of susceptibility to the action of toxic substances produced by the organism.

If local resistance were weak, though some of the organisms at the site of injection might be phagocyted, many would in the course of a few hours find their way through the lymph paths in the vicinity and become widely spread in the lymphatics and tissue spaces. Multiplication would ensue with only a short lag, particularly if the organisms were introduced at a favourable stage of growth. Toxic substances would be produced which in a few hours would reach a concentration sufficient to cause symptoms in an individual susceptible to their action. Some organisms would also get into the bloodstream, but the toxic bodies would doubtless be produced chiefly in the tissues. In resistant individuals many more organisms would be dealt with locally by the phagocytes and the area of inoculation would be rapidly walled off with strong inflammatory reaction. The organisms carried off in the lymphatics would possibly fail to multiply and ultimately be destroyed. Production of toxic substances would be slower and more localized.

It is inconceivable that sufficient staphylococcal toxin could be produced in vitro in the toxin-antitoxin mixture to account for the symptoms. Massive production of toxic substances "in vivo" must have taken place in the fatal cases if staphylococci were the responsible agents. The cases in which reactions were produced should have survived while in the fatal cases local reaction should have been slight or unnoticeable. This appears to have been the case in the Bundaberg fatalities. The lesions at autopsy would be those of an intense toxaemia, with indications of lymphatic involvement, as indeed they were.

The post-mortem findings in the Bundaberg children are strictly in accord with toxaemic death and are in no sense specific. Apart from cloudy swelling in the liver and kidneys, the changes in the lymphatic system are the most distinctive. In the lymph glands at the root of the lung and in the porta hepatis there are polymorph infiltration of the afferent lymphatic paths, and conspicuous proliferation of the endothelium of the dilated lymph sinuses, the proliferating cells exhibiting active phagocytosis. The malpighian corpuscles of the spleen also exhibit central degenerative changes affecting lymphocytes and endothelial cells. There are no infarctions, but the deaths occurred so rapidly that none could reasonably be expected to have occurred even had septicaemia been present.

The thrombus from the inferior vena cava of J.P. (the only example examined by the Commission) was unquestionably ante-mortem and possibly those described in the other cases were of the same nature. We have found no evidence of endothelial damage in the walls of the large veins in W.F. and T.R. in which the thrombi were described as being present.

We hesitate to draw conclusions from the distribution of the organisms in the blood and tissues of the cases examined post-mortem. In the only case in which staphylococci were found in abundance, other organisms were present which probably originated from the intestine and gained access to the blood stream in the terminal stages of the illness or entered the vessels post-mortem, though apparently unmistakable single and paired cocci were found in lymph glands and spleen in this and other cases. Any deductions which might be drawn from their presence in small numbers, even in suggestive surroundings, must necessarily be open to grave suspicion owing to the fallacies involved in such an examination.

Were it assumed that the deaths at Bundaberg occurred from fulminating staphylococcal septicaemia, they should in theory have been spaced out over a longer period and in those individuals who survived, the course of the disease should have been more prolonged and attended by evidence of metastasis. The distribution of the deaths and the lengths of the illnesses in the survivors at Bundaberg may be regarded as an expression of the degree of immunity of the individuals at the time of inoculation. Apart from possible congenital differences, this should show some correspondence with age. So far as we are aware there is no careful analysis of the influence of this last factor in staphylococcal infections in childhood.

With the doubtful exception of the pleurisy in the case of W.S. and the equally dubious abscess in the buttock in E.D., there were no metastases in the Bundaberg cases. Further, in three of the survivors, blood cultures made on the fourth day, when, it is true, only one of these children had pyrexia, and that of slight degree, were sterile. These facts make it clear that staphylococci were not present in the blood of the survivors for any length of time.

There is, therefore, no evidence that any of the survivors had septicaemia, nor do we suggest that this was necessarily the cause of death in the fatal cases. We present instead the hypothesis that the deaths resulted from an overwhelming toxaemia at an early stage of the invasion of the organism; that in the survivors staphylococci, if present in the blood stream at all, were few in number and failed to multiply to any extent.

Whether or no there were septicaemia in the fatal cases we have no clear evidence upon which to form an opinion. In only one case (M.Br.) at autopsy, 52½ hours after death, was a blood culture obtained. Only a few colonies of putrefactive organisms were found when the culture was plated, but we are not inclined to lay any stress on this observation because we found organisms in clot from cases autopsied at a much shorter period after death. The clot in the right auricle in W.F. and the ante-mortem thrombus from the inferior vena cava of J.P. both contained large numbers of cocci.

Possibly the negative findings recorded by Dr. Richards are explained by the fact that most of the organisms present in the blood had become entangled in the clot.

On the other hand it may be contended that the slowness of development and nature of the abscesses in the survivors, together with the very slight symptoms attendant upon their "ripening," point to the low pathogenicity of this organism for man. If it be assumed, upon this wholly insufficient evidence, that the Bundaberg staphylococcus is a relatively harmless organism capable only of producing a local lesion, the deaths at Bundaberg, which are so certainly related to the injections, must have been due to the presence in the toxin-antitoxin mixture either of an unknown toxic substance or of some living agent undemonstrable by the methods we employed, the only criteria of whose presence are the results of injection into young human beings. The absence of symptoms in children injected on occasions before the 27th January must be held to demonstrate its absence from the mixture at those times and to suggest that, like the staphylococcus, it was introduced during the course of the earlier inoculations.

Apart from the preconceived opinion that pyogenic staphylococci are incapable of causing rapidly fatal results when injected in large numbers into young children, there is no necessity to postulate such a hypothetical agent.

We are, therefore, of the opinion that the deaths at Bundaberg were caused by the injection of living staphylococci in toxin-antitoxin mixture, a conclusion which rests in part upon negative evidence, other possible causes of the disaster having been excluded.

Short of the unthinkable experiment of injecting living staphylococci into children, it is not possible to offer rigid proof. The positive evidence is by itself suggestive rather than conclusive and is briefly summed up as follows :—

(1) Abscesses occurred at the site of the injection in all the surviving children who were inoculated on 27th January at Bundaberg. From five which were examined, staphylococci identical with those present in the original bottle were recovered in pure culture.

(2) The symptoms exhibited by the children who died conformed to the general picture of fulminating staphylococcal infections.

(3) The post-mortem findings in the children who died are compatible with extremely rapid death from an infecting organism.

(4) The staphylococcus in question is capable when grown "in vitro" for eight hours of yielding a toxin which produces positive and specific skin tests in a dilution of 1 in 2,000 in susceptible individuals. Further, the reactions are intensified when the organism has been grown in media to which human blood has been added. Only a proportion of the individuals tested are susceptible to the toxin even in high concentrations.

(5) Cultures of the Bundaberg staphylococcus in toxin antitoxin mixture exhibit variations in activity when injected into animals at varying times which fit the observed facts in regard to this tragedy.

There are the following difficulties in accepting this view :—

(1) Staphylococcal infections are generally local and when septicaemia occurs it is usually less rapidly fatal than were the cases at Bundaberg. (Natural infections with staphylococci on account of the small numbers of organisms usually introduced do not provide a parallel case and consequently clinical evidence that staphylococci do not usually cause death in so short a time cannot be held to exclude this possibility.)

(2) From clinical experience it might be predicted that had the children at Bundaberg died from fulminating staphylococcal infection, the cases which recovered should have shown definite metastatic abscesses. (There are only the doubtful abscess in E.D. and the pleurisy in W.S.).

(3) The absence of gross lesions at the autopsies apart from ante-mortem thrombi in the great vessels might be held to exclude staphylococcal septicaemia, save that the time from the onset of symptoms to death was too short for lesions to be produced. (Instances of acute staphylococcal septicaemia are quoted in the text in which no lesions were found at autopsy; similarly rabbits dying rapidly after intra venous injection may show no lesions. (Appendix 23.)).

(4) In the single autopsy 52½ hours after death, staphylococci were not recovered from the blood culture. Sterile blood cultures were also obtained on the third and fourth days in children who recovered.

(5) The relative non-virulence of the organism to laboratory animals with the exception of rabbits. (This finding is common, according to Vincenzi, in acute staphylococcal infections.)

Looking at the unfortunate accident at Bundaberg as a crucial experiment on the infectivity and virulence towards young children of staphylococcus aureus growing in a fluid medium, we are not aware of any precedent or of any parallel experiment. On some day between 17th and 24th January—and most probably on the 24th—the bottle of toxin-antitoxin became contaminated by the Bundaberg staphylococcus. It was incubated for at least three days at Bundaberg temperatures, and on the 27th eight children received subcutaneously four minims, of whom six died and two were ill, but recovered, afterwards developing local abscesses, and also thirteen received two minims, of whom six died, four were ill and afterwards developed local abscesses and three were not ill, but developed abscesses. The disastrous result of this fortuitous experiment is not what we ourselves would have expected, and will doubtless occasion surprise and possibly controversy, but we have found ourselves gradually driven to this verdict by the accumulation of evidence, both positive and negative, incomplete though it be. Owing to the absence of bacteriological investigations during the acute illness and the very inadequate post-mortem examinations, we cannot assert that an overwhelming septicaemia occurred in the fatal cases.

The variations in susceptibility disclosed at Bundaberg, viz., from death in sixteen hours to a local abscess are almost paralleled in natural human diseases, e.g., diphtheria, pneumonia, epidemic influenza. We have said " almost " because there are two striking differences between most natural infections and the Bundaberg cases. They are the extremely short incubation period and the rapid progress to a fatal issue. We have, however, adduced a reason for these differences. Our hypothesis demands that a very large number of organisms was directly introduced into the subcutaneous tissues of extremely susceptible, partially susceptible, and almost insusceptible children. An incompletely sterilized needle introduces but a few organisms and an abscess may result. An accidental wound or an operation area is contaminated with but a few organisms and sepsis may result. In these instances there is time for the development of the local immune mechanism. But the sudden introduction of a large number, in all probability several millions according to our conservative calculations, would be received by unprepared subcutaneous tissues, and it is inconceivable that some would not within a very short time reach the general circulation. Whether a temporary bacteriaemia or a septicaemia would result might then depend, not on the local immune mechanism, but on " humoral " immunity.

The Source of the Contamination.—We have attempted to ascertain the source of the contamination in Dr. Thomson's bottle. All the unopened bottles of toxin antitoxin without added antiseptic (Batches 78A and 86) which we examined have been perfectly free from any turbidity and all those which we have cultured have proved to be sterile (Appendix 16). There can be no suggestion that the contamination had already occurred when the bottle in question left the Commonwealth Serum Laboratories in November. We are thoroughly satisfied with the technique of filling and with the checks upon the sterility of the biological products issued from the Laboratories. Thorough inspection for cracks in the containers and for specks or particles suggestive of possible air-borne contamination is made and any ampoule or bottle of which there is any suspicion is discarded. Neither is there any question that when Dr. Thomson's bottle reached him on 7th January, it was still uncontaminated and indeed, according to Dr. Thomson's evidence, the contents remained clear till the evening of 27th January. In the meanwhile it was stored in a cupboard in his surgery and remained in its carton unopened till 17th January. It was used for inoculations on the 17th, 20th, 21st, 24th, and 27th and between these times of use it was stored in the doctor's surgery at a temperature which probably varied between 70° and 80° Fah.. (Appendix 7). It is certain that contamination occurred on one of these occasions when toxin-antitoxin mixture was being withdrawn from it.

Dr. Thomson's Technique.—We have inquired into Dr. Thomson's technique in withdrawing samples for inoculation and in addition he has been good enough to demonstrate his method for us in the room where most of the inoculations were done (Appendix 3).

This demonstration, carried out on the afternoon of 15th February, consisted in the withdrawal of two samples of 1.0 c.cm. of toxin-antitoxin mixture from two bottles of Batch 78A and one of Batch 86 (containing no antiseptic). We find that there are two ways in which contamination may have occurred.

As described in Appendix 3, Dr. Thomson, after thoroughly scrubbing his hands with soap and water, fixed the needle to the nozzle of the syringe, picking it out of sterile water with his fingers instead of carrying out this operation with a pair of sterile forceps. The contact of Dr. Thomson's fingers with the water in which the needles were allowed to lie, in order to remove the methylated spirit in which they had been sterilized, gave opportunity for the contamination of this water and hence of later needles withdrawn from it.

Air Contamination.—The second possibility of contamination arises from the fact that it is not possible to withdraw material satisfactorily from rubber-capped bottles without first admitting a corresponding quantity of air. When the air in the room is still, it is possible with rigid technique to withdraw samples or introduce air into the bottles of toxin-antitoxin mixture without contaminating them. If the air in the room is in motion, as it was as a result of the use of a fan on all the occasions when Dr. Thomson carried out inoculations and when he demonstrated his technique for us, even with rigid technique it is impossible to avoid contamination (Appendix 4). Of the three bottles in the experiment Dr. Thomson made for us two were contaminated, one containing the only staphylococcus recovered from any of our air contamination experiments or in the examination of partly used bottles of toxin-antitoxin mixture which at all resembles the Bundaberg staphylococcus.

On our return to Melbourne we carried out a further experiment in which we used a powerful fan to cause air movement and injected large quantities of air so as to provide every opportunity for air contamination. We used two sets of bottles of toxin-antitoxin mixture, one with, and one without, antiseptic. In this way we successfully contaminated all of four bottles containing no antiseptic, but bottles containing antiseptic subjected to similar treatment have remained

perfectly clear and we have not been able to recover any organism from them by culture. Further, our attempts to grow the Bundaberg organism in toxin-antitoxin mixture containing antiseptic have invariably failed, even when heavy inocula have been used.

It is certain then that when antiseptic is present in the mixture the possibility of air-borne contamination, the only serious possibility with good technique, is eliminated.

We have no clear evidence as to whether Dr. Thomson's bottle was infected by air contamination or by contamination of the water in which the needles were immersed. We think the latter is the most probable explanation as far as the staphylococcus is concerned, since serologically it is unrelated to the common air-borne cocci which we obtained in several of our air contamination experiments.

The Withdrawal of the Sample from Dr. Thomson's Bottle.—At this stage it is important to discuss the question of the withdrawal of a sample from Dr. Thomson's bottle of toxin-antitoxin mixture, admittedly already turbid, by Dr. McKeon on 28th January. This was done with a sterile syringe and needle, but the top of the rubber cap was not sterilized. It may be argued that this in itself vitiates our examination of the residual contents of the Bundaberg bottle.

The bacteriological examination of the contents of the original bottle and of the sample withdrawn by Dr. McKeon showed that both contained a staphylococcus identical with that isolated from the abscesses at the site of inoculation in the survivors. As far as the case against this staphylococcus is concerned, therefore, the omission by Dr. McKeon to sterilize the top of the bottle does not in the least affect our conclusions.

Since we were not able to grow any other living organism, pathogenic or otherwise, either from the contents of the original bottle or the portion withdrawn, the faulty technique of the withdrawal of the sample is without significance.

Investigation of other partly-used Bottles.—The use by other medical practitioners of material of Batch 86 is of importance in the present inquiry. In Appendix 17 we have recorded the results of testing the contents of a number of partly used rubber-capped containers of this batch, together with the manner in which the material was used. It will be observed that a number of capable practitioners used them in the same way as did Dr. Thomson, making injections on more than one occasion at varying intervals of time from the same bottle. This practice doubtless arose from the fact that vaccines containing antiseptic are commonly so used. With the exception of vaccines which are specifically prepared for use on a number of occasions, biological products should, however, be used up as rapidly as possible after opening them, and the unused balance, if any, discarded. A fortiori, in a tropical or sub-tropical climate this rule should be observed, since the growth of any organisms accidentally introduced may be rapid.

The Absence of Antiseptic in Batch 86. Narrative re the Warning Notice.—When Dr. Morgan, Director of the Laboratories Division, Commonwealth Health Department, decided to avoid the possible risks attendant upon freezing toxin-antitoxin mixture, by leaving out the antiseptic, his intention was that all future samples of the product should be issued in sealed glass ampoules to avoid any risk of further use of the mixture after it had been opened and possibly contaminated. This method of putting up the product is somewhat inconvenient for use when large numbers of injections are to be given at one time. Requests were made to Dr. Morgan for rubber-capped containers of the mixture for this purpose. Batch 78, the first batch made up without antiseptic, was issued only in sealed glass ampoules. It was ready for issue on the 25th August, 1927. The next two batches, 86 and 78A, were bottled on the 10th October, and the 28th November, 1927. Both were put up partly in ampoules and partly in rubber-capped containers. Dr. Morgan was careful to warn Dr. Hilda Bull, who was doing large scale immunization for the Melbourne City Council, and who had specifically asked for rubber-capped bottles, that the mixture would shortly be issued without antiseptic and, therefore, should be used up at once, any unused material being discarded.

The first issue of Batch 86 was of 24 rubber-capped containers to the Commonwealth Health Department, Brisbane, and was made in response to an emergency order on 12th October, 1927. On about 22nd or 23rd November, 1927, Dr. Morgan decided that it was advisable to warn specifically practitioners using this product, that it contained no antiseptic and must not be used on a number of separate occasions like a vaccine. This notice was printed and ready for enclosure with the rubber-capped containers of Batch 78A on 19th December, 1927. Between 30th November and 19th December, 1927, a typewritten copy of the notice was sent out wrapped round every package and fixed with a rubber band.

In the meanwhile in response to a request for rubber-capped bottles in exchange for ampoules in stock at the Brisbane Office, Dr. Morgan sent a copy of this notice to Dr. Elkington on 25th November, 1927, with a covering letter which unfortunately did not make it clear that

the second issue of thirty rubber-capped bottles of Batch 86 and eighteen rubber-capped bottles of Batch 78A, sent off to the Brisbane Office on 29th November, 1927, would not contain this warning notice, which was not yet available in printed form. From the last sentence of Dr. Morgan's letter (Appendix 15), Dr. Elkington justifiably drew the conclusion that future issues of the material would contain the warning notice inside the carton. Dr. Elkington gave instructions that mimeographed copies of this circular were to be issued with all the accounts in December to firms who retailed the products and to any medical practitioner who purchased directly, but in view of Dr. Morgan's letter did not consider it necessary to circularize the medical profession in Queensland.

The only issues directly from the Divisional Office, Brisbane, after Dr. Morgan's letter was received, were to the Murgon Shire Council (material presumably used by Dr. Randall). The remainder were made through Queensland Druggists Ltd. (Dr. Green and Dr. Reye's bottles), and through Medical and Surgical Requisites Ltd. (Dr. Thomson's bottle). Though the firm of Taylor and Elliotts and Doctors McLean and Earnshaw received a typewritten copy of the warning notice early in January, 1928, the Manager of Medical and Surgical Requisites Ltd., Brisbane, denied having received it from the Commonwealth Health Department, Divisional Office, Brisbane, and in any case it is clear that it was not transmitted to Dr. Thomson.

History of Diphtheria.—In his evidence before the Commission Dr. Cumpston sketched the history of diphtheria in Australia and made some important comments on the problem of its control. In general the death rates from diphtheria in the various States were already declining before the introduction of antitoxin in 1895, though notification indicates that the disease is still active in the community. The diphtheria death rate after 1890 shows a general parallelism with the birth rate. Tracing a correspondence between immigration rate and diphtheria incidence, with the experience of Western Australia in the years following the gold discoveries as a striking example, Dr. Cumpston suggests as potent factors in the influence of immigration on diphtheria incidence, the disturbance of the composition of the population in its adaptation to the disease, the increase of births and improved means of communication. He points out that the period 1921 to 1931 promises to show the greatest influx of population as yet experienced in Australia, which, in spite of the declining birth rate, is likely to cause a definite activity of diphtheria over several years.

The value of active immunization as a prophylactic against diphtheria is a question of public health which does not come strictly within the scope of this inquiry.

The fatalities at Bundaberg occurred incidentally in the early stages of a campaign of active immunization against diphtheria. This was a public measure carried out on medical advice necessarily based on experience gained in other countries. In Australia, immunization has been limited in scope and confined to institutions, to sporadic municipal ventures, and to private prophylaxis. No authoritative pronouncement can, therefore, be made from evidence gained from Australian sources alone.

It should be emphasized that active immunization is the only specific measure at present known for the control of the incidence of the disease, and that there is no inherent danger in its practice when properly controlled.

FINDINGS.

1. The injections of toxin-antitoxin mixture administered by Dr. Thomson at Bundaberg on 27th January, 1928, were responsible for the deaths of twelve out of twenty-one children inoculated and for the illness of several who survived.

2. The toxin-antitoxin mixture as issued by the Commonwealth Serum Laboratories was properly prepared and sterile, but contained no antiseptic and, therefore, did not prevent the growth of micro-organisms accidentally introduced into it.

3. The rubber-capped bottle of toxin-antitoxin supplied to Dr. Thomson at Bundaberg was accompanied by no information as to the presence or absence of antiseptic.

4. The omission of antiseptic was intended to safeguard against the dangers attendant upon freezing, but the issue of this preparation without antiseptic in rubber-capped bottles suitable for repeated usage is an unsound procedure.

5. The toxin-antitoxin mixture in the bottle used by Dr. Thomson was contaminated by him with a pathogenic staphylococcus during a series of inoculations on 17th, 20th, 21st and 24th January, most probably on the last occasion.

6. The consideration of all the available evidence concerning the deaths at Bundaberg points to the injection of living staphylococci as the cause of the fatalities.

RECOMMENDATIONS.

1. That biological products in which the growth of pathogenic organisms is possible should not be issued in rubber-capped containers for repeated use unless there is present in the material a sufficient concentration of antiseptic to inhibit bacterial growth.

2. That all biological products not containing antiseptic, however issued, should bear a conspicuous printed notice, both on the container and on the package, to the effect that no antiseptic is present; that they should be used immediately on opening, and any remaining product discarded.

3. That biological products, should be distributed in bottles or ampoules of clear glass in which it is easier for the medical practitioner himself to detect turbidity or any other defect.

4. That the Commonwealth Department of Health should make full and careful enquiry as to whether it be advisable to substitute anatoxin or some similarly modified immunizing agent for toxin-antitoxin.

5. To ensure the adoption of the most approved technique, facilities for special post-graduate training should be afforded to all medical officers entrusted with the conduct of public campaigns to diminish the incidence of disease by immunization.

In concluding this report we desire to acknowledge the courtesy and assistance we have received from individuals, officials and institutions in the course of this enquiry. We wish to record our appreciation of the assistance rendered to us, particularly in making materials available, by the Director-General of Health of the Commonwealth and the Director of the Laboratories Division of the Commonwealth Health Service. Our thanks are due to the Staffs of the Bundaberg General Hospital, of the Pathological Laboratories of the Brisbane and South Coast Hospitals Board, and of the Royal Prince Alfred Hospital, Sydney. We also desire to express our special indebtedness to the Trustees and Board of the Walter and Eliza Hall Institute for allowing us to carry out these investigations here, and to the Staff of the Institute who have assisted in many aspects of the investigation, particularly to Dr. F. M. Burnett, Mr. H. F. Holden, and Miss F. E. Williams. Finally our thanks are due to Sir Henry Maudsley and Dr. R. R. Stawell who discussed with us the clinical aspects of the problem.

We have the honour to be,

Your Excellency's most obedient Servants,

CHARLES H. KELLAWAY, Chairman.

P. MacCALLUM,

A. H. TEBBUTT.

R. A. REID, Secretary,

Melbourne, 11th June, 1928.

APPENDICES.

1. Map of Bundaberg and its environs, showing the location of the homes of children inoculated on 27th January.

2. Description of the room in which the injections were performed by Dr. Thomson and where experiments on air contamination were carried out by the Commission. (With figures 1, 2 and 3.)

3. Demonstration by Dr. E. G. Thomson of the methods used by him in withdrawing toxin-antitoxin mixture from rubber-capped bottles.

4. Air contamination experiment carried out at Bundaberg.

5. Examination of the record syringe and needles used by Dr. E. G. Thomson at Bundaberg.

6. Examination of the boiled rainwater used by Dr. E. G. Thomson on the 27th January, 1928.

7. The temperature at which Dr. E. G. Thomson's bottle of toxin-antitoxin mixture was stored.

8. The treatment of the cases; and temperature charts from the Bundaberg General Hospital.

9. Detailed narrative of events in relation to the request of the Mayor of Bundaberg for a pathologist.

10. Dr. Richards reports on the autopsy of M. Br.

11. Detailed description of the pathological findings.

12. Analysis of organs for heavy metals.

13. The preparation of Batch 86—diphtheria toxin-antitoxin mixture.

14. Disposal of Batch 86.

15. Warning notice and covering memorandum addressed to Dr. J. S. C. Elkington from Dr. F. G. Morgan.

16. Examination of unopened bottles of Batch 86 and of other batches without antiseptic.

17. Results of investigation of partly used rubber-capped bottles of Batch 86 with their previous history.

18. Examination of the remainder of the toxin-antitoxin mixture in the bottle of Batch 86 from which Dr. Thomson made his injections on the 17th, 20th, 21st, 24th and 27th of January.

19. Examination of the Bundaberg bottle of toxin-antitoxin mixture for iodine.

20. On the possibility of tetanus being the cause of the fatalities.

21. Is it possible that organisms other than staphylococci were present in the Bundaberg bottle of toxin-antitoxin mixture, which had died out or been destroyed by the proteolytic activity of the staphylococci?

22. Report of serological and other characteristics of staphylococci associated with the Bundaberg Investigation.

23. The pathogenicity of the Bundaberg staphylococcus to laboratory animals.

24. The production of a skin-reacting toxin from the Bundaberg staphylococcus.

25. The effect of the Bundaberg staphylococcus on toxin and antitoxin separately.

26. On the cultivation of staphylococci in toxin-antitoxin, on the possibility of dissociation of toxin by this method, and on the pathogenicity of staphylococci grown in this medium.

27. On the development of turbidity in toxin-antitoxin mixture in relation to the number of staphylococci in Dr. Thomson's bottle on 27th January, 1928.

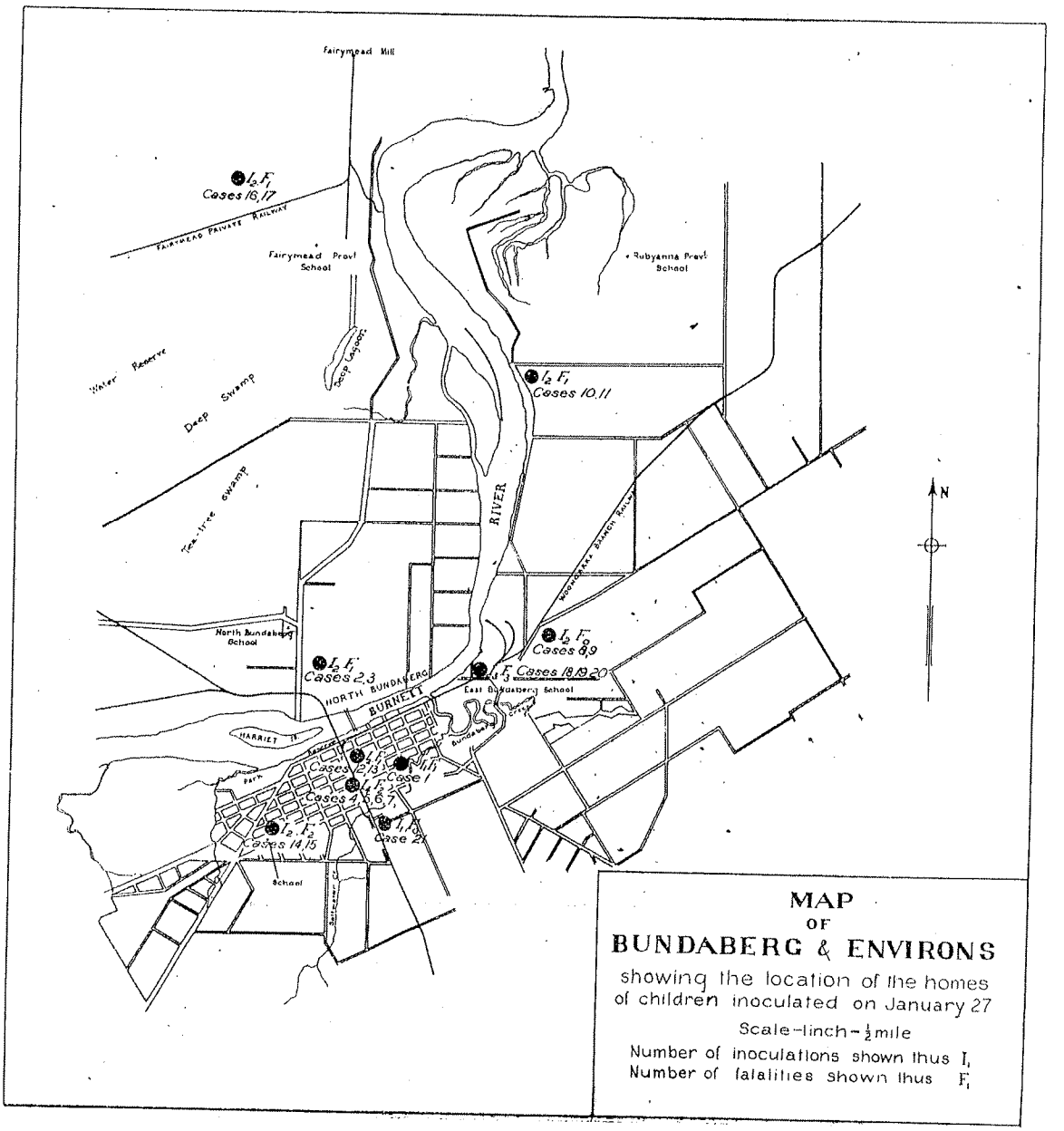
28. Investigation of the content of diphtheria antitoxin in the sera of the survivors of the injection of toxin-antitoxin mixture on 27th January, at Bundaberg.

29. On the effect of subcutaneous injection of diphtheria toxin together with the Bundaberg staphylococcus.

30. On generalized staphylococcal infections.

31. Definition of some technical terms used.

APPENDIX 1.


Fairymead Mill
½ F1
Cases 16,17
FAIRYMEAD PRIVATE RAILWAY
Fairymead Prov! School
Rubyanna Prov! School
Water Reserve
Deep Swamp
Deep Lagoon
½ F1
Cases 10,11
RIVER
Tea-tree swamp
North Bundaberg School
½ F1
Cases 2,3
½ F2
Cases 8,9
½ F3 Cases 18,19,20
East Bundaberg School
NORTH BUNDABERG
BURNETT
HARRIET ST
Cases 2,3
Case 1
Cases 4,5
½ F2
Cases 14,15
½ F1
Case 21
School
N
MAP
OF
BUNDABERG & ENVIRONS
showing the location of the homes of children inoculated on January 27
Scale–1inch–½mile
Number of inoculations shown thus I1
Number of fatalities shown thus F1

APPENDIX 2.

DESCRIPTION OF THE ROOM IN WHICH INJECTIONS WERE PERFORMED BY DR. THOMSON AND WHERE EXPERIMENTS ON AIR CONTAMINATION WERE CARRIED OUT BY THE COMMISSION.

This room, the Engineer's Office, which is placed at the rear of the Council Chambers, away from the main street, has one door and one window on the northern aspect. (Fig. 1). A third window was not obvious from the interior as the western end of the room was occupied by stretchers piled up to the roof and harbouring a good deal of dust. The door and northern window look out on a small grass covered yard at the rear of the building which is continued on its eastern side, but which is not open to the street. Entry is effected to the room through the Council Chambers.

The room has a concrete floor 12 x 18 feet, but only about two-thirds of this space was available, as the stretchers and other oddments stored there were not disturbed when it was cleaned out for Dr. Thomson's use. A table and two chairs occupied the central space (Figs. 2 and 3), and there was also a high stool on which the basin " B " stood. Along the eastern wall was a long desk " M." The chair seen in the photograph (Fig. 3) is the one at which the doctor sat.

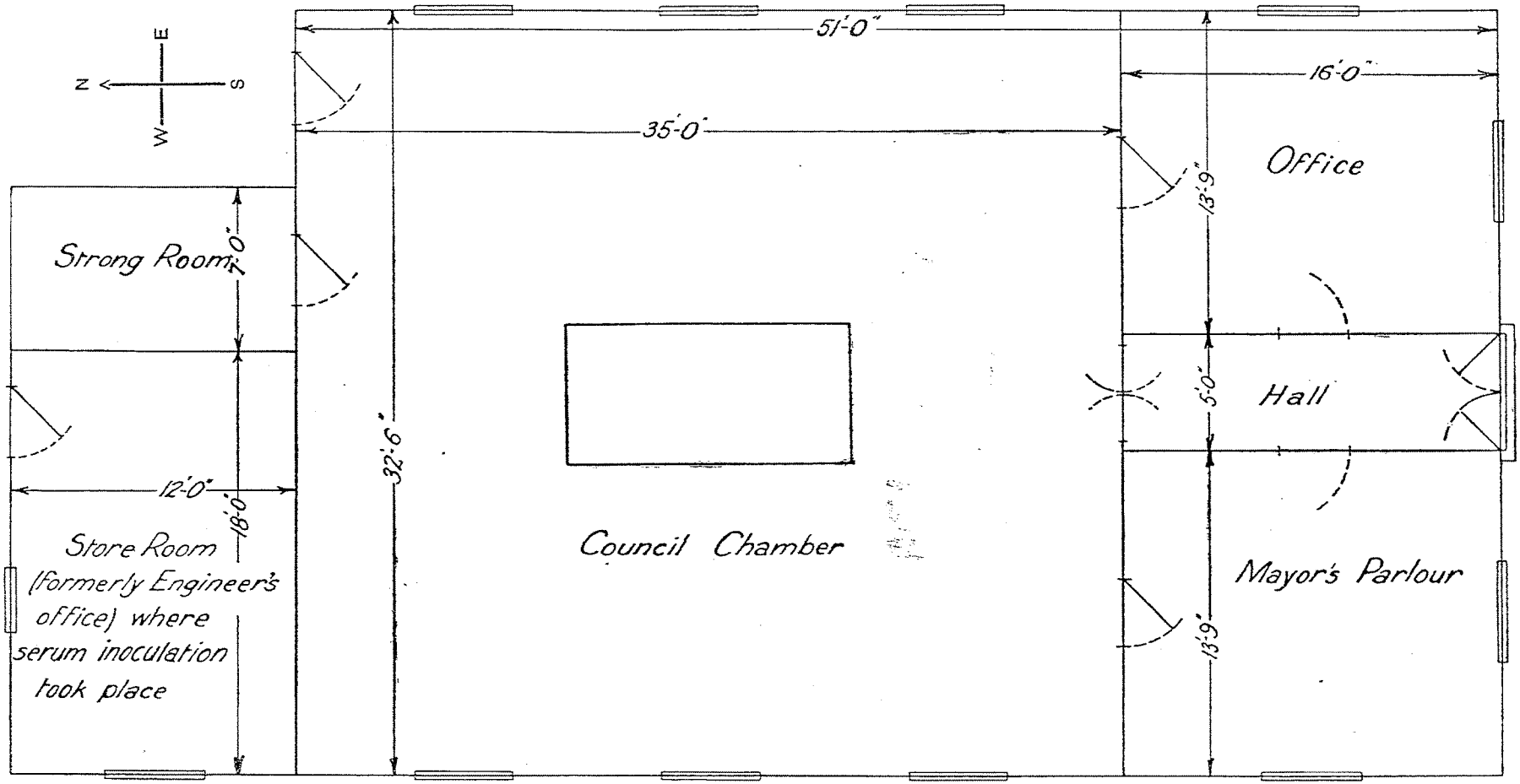


FIG. 1.

SKETCH PLAN OF COUNCIL CHAMBERS, CITY OF BUNDABERG.

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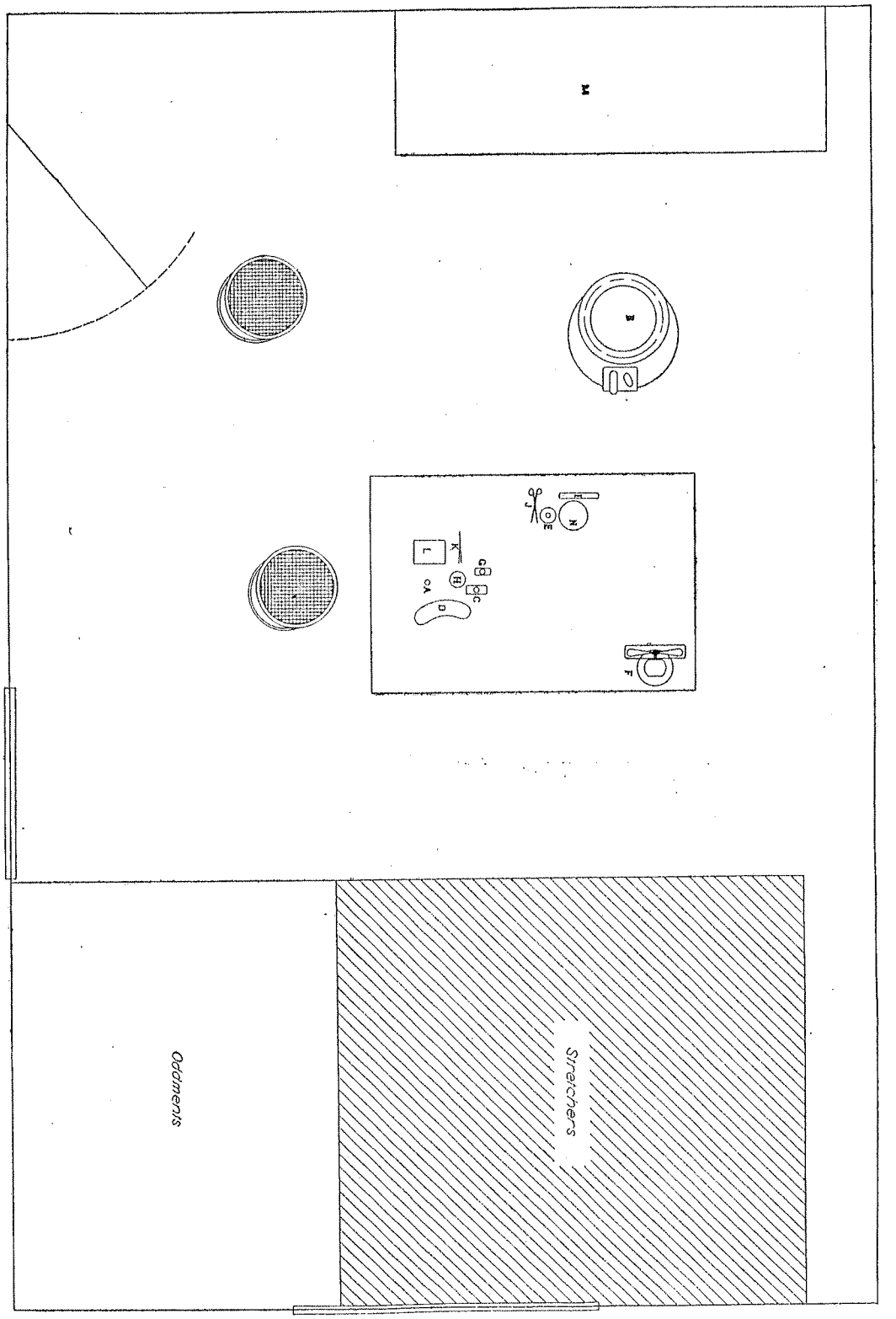
FIG. 2.



GROUND PLAN OF ROOM WHERE DR. E. G. THOMSON CARRIED OUT HIS INOCULATIONS.

A. Small conical glass vessel containing methylated spirits for sterilization of needles.
B. Basin where Dr. Thomson washed his hands before making the injections.
C. Bottle containing boiled water.
D. Kidney dish containing boiled water for immersion of needles after sterilization in methylated spirit.
E. Reel of Z.O. plaster.
F. Electric fan.
G. Bottle containing methylated spirits.
H. Large conical glass measure containing methylated spirits and swabs for cleaning up the children's arms for injection.
I. Iodine pencil.
J. Scissors.
K. Forceps.
L. Syringe and needles in case.
M. Desk along side of room.
N. Basin for used swabs.

APPENDIX 3.

DEMONSTRATION BY DR. E. G. THOMSON OF THE METHODS USED BY HIM IN WITHDRAWING TOXIN-ANTITOXIN MIXTURE FROM RUBBER-CAPPED BOTTLES.

On 15th February, Dr. Thomson and his nurse arranged the furniture in the Engineer's room at the Municipal Chambers as it had been on 27th January, and laid out on a small table covered with a clean sheet the apparatus used for inoculations in approximately the positions which they occupied when the inoculations were performed there. Part of the interior of the room and the arrangement of the table and the objects on it are shown in the photograph taken at 4 p.m. immediately before Dr. Thomson's demonstration to the Commission (see Figs. 2 and 3). The door was widely open and the north window in its lower half, as on 27th January, and the preceding occasions when inoculations were performed. The position of the fan on the table is shown in the photograph. It was in action during the procedures to be described, as it was on all the occasions when Dr. Thomson performed inoculations. The draught from it blew directly across the far end of the table and not over that portion where the materials for inoculation were laid out.

At 4.10 p.m., in the presence of the Chairman and Nurse I. G. Currey, Dr. Thomson drew off two successive samples of 1 c.cm. from each of three rubber-capped bottles of batches 78A and 86 which contained no antiseptic.

His technique was briefly as follows :—

The syringe and needles, enclosed in their metal case, were brought over from Dr. Thomson's rooms where they had been sterilized, by boiling for fifteen minutes and the needles, four in number, were now placed in the vessel " A " in methylated spirits.

The syringe was a 1 c.cm. glass and metal " Record " graduated in tenths of a c.cm. and in minims and the needles used were four in number. The syringe and needles were those recently used by Dr. Thomson, and in the intervening period examined by the Commission (Appendix 5).

Dr. Thomson first scrubbed his hands with soap and water in the basin " B," using a nail brush and drying them on a freshly laundered hand towel. He then transferred the needles from methylated spirits in " A " to the kidney dish " D " containing boiled water from the bottle " C." This was done by hand. A needle was now taken from the water also by hand and fitted to the syringe, water being expelled from the syringe and needle by forcing down the piston. Air was now drawn into the syringe. The centre of the rubber cap had been moistened with iodine solution from an iodine " pencil " (I.), and now the needle was passed through this part of the rubber cap, air was forced into the bottle and a sample of toxin-antitoxin mixture was withdrawn. This was expelled from the syringe, the needle was placed in the methylated spirits in vessel " A " and a fresh needle was taken by hand from the kidney dish " D " and fitted to the nozzle of the syringe. Air was again taken into the syringe and the procedure was repeated. When the needles had all been used once they were taken from the methylated spirits in which they had been immersed for two minutes or so, and replaced in the kidney dish " D " in water. From this the needles necessary to complete the experiment were taken.

Each of three bottles, therefore, had inoculated into it 2 c.cm. of air in order to withdraw the corresponding amount of toxin-antitoxin mixture.

The bottles were not incubated but kept packed in cotton wool in a closed wooden box, which was kept in a closed suit case and in this way were maintained at approximately the temperature of Bundaberg for a few days.

The subsequent history of these three rubber-capped bottles is as follows :—

T.1. No turbidity after two days—sterile when tested by culture after a week. Guinea pigs inoculated each with 1.0 c.cm. on the fourth and seventh days showed no infiltration and survived.

T.2. Turbid after two days—sub-culture by plating on blood agar on the seventh day showed the presence of a faintly haemolytic staphylococcus albus which Dr. Burnet, of the Hall Institute, was able to show did not correspond to the organism isolated from the fatal bottle, also a non-haemolytic staphylococcus albus and a Gram-negative cocco-bacillus. Guinea pigs inoculated with 1.0 c.cm. of the mixture on the fourth and seventh days showed no infiltration and survived.

T.3. was also turbid after two days and from it on the seventh day a faintly haemolytic staphylococcus albus like that in *T* 2 was isolated. Guinea pig experiments on the fourth and seventh days were negative.

agar plates, one loopful only being used. The subcultures from the bottles of batch 111, containing antiseptic showed no growth even after incubation for eight days at 37°. Those from the bottles without antiseptic all showed growth in 24 hours, and stroke cultures on agar plates gave numerous colonies in three, but only a few in the fourth.

The results of subculture on agar plates are summarized below—

Bottle 1. Many white colonies of staphylococci.
„ 2. Many yellow colonies of staphylococci and white colonies like those of 3.
„ 3. Staphylococci forming large flat white colonies with wavy margins. Many colonies.
„ 4. Four yellowish colonies of tetracocci, one mould and three large colonies like those of 3.

It is evident that the presence of antiseptic, despite statements given in evidence to the contrary, is of considerable value in preventing air-borne contamination—a result which is in accord with experience in the use of vaccines containing antiseptic.

We attempted also to grow the Bundaberg staphylococcus (Appendix 18) in bottles of batch 111 containing 0·5 per cent. phenol, but were unable to do so, using small inoculations which readily produce growths in rubber-capped bottles without antiseptic.

APPENDIX 5.

EXAMINATION OF THE RECORD SYRINGE AND NEEDLES USED BY DR. E. G. THOMSON AT BUNDABERG.

These were received in their metal case on 7th February. The glass barrel was somewhat cloudy looking. There were six (6) needles, four (4) marked R.S. (rustless steel), two (2) with no such marking. One (1) R.S. needle had no stylet in it, three (3) R.S. needles and the other two contained stylets.

A special examination was immediately carried out. Another sterile syringe fitted with a needle was taken and sterile saline drawn in and out several times, and then a little saline was drawn up, the plunger drawn backwards and forwards several times and the fluid ejected on to a slide.

The Bundaberg syringe was fitted together and the same needle as above (after being washed out again with the other syringe) fitted to it, a minute amount of saline drawn up and the inside of the barrel washed with it. It became obviously turbid and was ejected into a sterile watch glass. A film of this was made and a stroke culture on plain agar. Saline was then drawn up into the syringe and ejected numerous times, the barrel becoming cleaner looking except a small section near the needle end which remained cloudy.

The needle without stylet, called needle No. 1, was then taken and the Hall Institute syringe fitted to it. A minute amount of saline was drawn up and ejected numerous times on to a sterile watch glass. The saline became markedly cloudy and flocculation occurred later in the watch glass. A film and culture were also made from this fluid.

All the other needles, Nos. 2 to 6, were treated in the same way. Only one gave a turbid washing and this was much less turbid than No. 1 needle.

Examination of the films in all cases showed only debris and no organisms. The cultures from the syringe and needle No. 1 gave no growth.

At the time these results were puzzling, but they became explicable after evidence was taken at Bundaberg. Evidence on oath was given that Dr. Thomson's nurse boiled the syringe and needles after their use on 27th January, and that subsequent to the fatalities Dr. McKeon removed a small amount from the bottles with this same syringe, transferred it to a small phial and then plunged the syringe and needle into methylated spirits. Hence the turbidity of the washings from the syringe and one needle. No explanation is forthcoming for the slight turbidity of the washings from one other needle.

APPENDIX 6.

EXAMINATION OF THE BOILED RAIN-WATER USED BY DR. E. G. THOMSON ON THE 27TH JANUARY, 1928.

The water used by Dr. Thomson in his inoculations was obtained from a cylindrical, concrete-lined galvanized iron tank of 800 gallons capacity, which received rain-water draining from the roof of his house by two open gutters.

The water in the bottle which had been left unchanged from the 27th January to the 13th February, 1928, when it was examined, was inoculated in doses of ·5 c.cm. and 1 c.cm. into two bottles, one of batch 86 and the other of batch 78A.

In both cases marked turbidity was evident on the 17th February and culture on the 22nd February demonstrated the growth of staphylococci forming white colonies which were shown later on not to be identical with the Bundaberg staphylococcus isolated from the bottle of toxin-antitoxin mixture from which Dr. Thomson made the injections on the 27th January, 1928. These water staphylococci were larger than pyogenic staphylococci, and did not agglutinate with the sera of rabbits immunized with the Bundaberg staphylococcus.

Guinea pigs were inoculated from each of these bottles with a dose of 1 c.cm. subcutaneously on the 19th and 22nd February. The injections produced no ill effects.

APPENDIX 7.

THE TEMPERATURE AT WHICH DR. E. G. THOMSON'S BOTTLE OF TOXIN-ANTITOXIN MIXTURE WAS STORED.

The bottle of toxin-antitoxin mixture was kept in a closed glass instrument cupboard in Dr. Thomson's surgery and we were only able to arrive indirectly at the temperature at which it had been kept by getting Dr. Thomson to keep a record of maximum and minimum temperatures in the instrument case itself for nine consecutive days in the month of February. The thermometer which was used was corrected for us by Mr. F. H. Holden, of the Walter and Eliza Hall Institute, Melbourne. The temperatures observed by Dr. Thomson and the corrected temperatures are given below, together with the official Bundaberg temperatures observed at the Post Office during the two periods, 17th January to 27th January, and 20th February to 28th February. These last we obtained through the courtesy of Mr. H. A. Hunt, the Commonwealth Meteorologist.

TABLE SHOWING THE MAXIMUM AND MINIMUM TEMPERATURES BETWEEN 20TH FEBRUARY AND 28TH FEBRUARY, OBSERVED AND CORRECTED IN THE CUPBOARD IN DR. THOMSON'S SURGERY, TOGETHER WITH THE OFFICIAL TEMPERATURES RECORDED AT THE POST OFFICE, BUNDABERG, ON THE SAME DAYS AND DURING THE PERIOD OF STORAGE OF THE BOTTLE OF TOXIN-ANTITOXIN AFTER ITS FIRST USE ON 17TH JANUARY, 1928.

Date.	In Cupboard.				At Post Office.		At Post Office.		
	Maximum.		Minimum.		Maximum.	Minimum.	Date.	Maximum.	Minimum.
	Obs.	Cor.	Obs.	Cor.					
February,							January,		
20th ..	82°	80°	76°	77°	85°	76°	17th ..	81°	66°
21st ..	78°	76°	74°	75°	84°	71°	18th ..	83°	70°
22nd ..	77°	75°	73°	74°	81°	72°	19th ..	77°	71°
23rd ..	75°	74°	71°	72°	81°	75°	20th ..	86°	69°
24th ..	76°	75°	70°	71°	81°	70°	21st ..	87°	72°
25th ..	74°	73°	70°	71°	86°	70°	22nd ..	90°	74°
26th ..	74°	73°	72°	73°	72°	68°	23rd ..	88°	74°
27th ..	78°	76°	74°	75°	76°	68°	24th ..	85°	75°
28th ..	77°	75°	74°	75°	82°	68°	25th ..	85°	56°
							26th ..	88°	68°
							27th ..	86°	68°
Mean ..	..	75°	..	73.7°	81°	71°	..	85°	69·4°

The temperature in the cupboard will be seen to have been remarkably constant, and in all probability the mean maximum and mean minimum temperatures between 17th January and 27th January did not differ appreciably from 79° and 72° F.

For the period 23rd January to 27th January, the mean official maximum and minimum Bundaberg temperatures were 86° and 68° F., and in our experimental attempts to produce toxicity in samples of toxin-antitoxin mixtures we have, therefore, used an incubator set at 80° F.

APPENDIX 8.

THE TREATMENT OF THE CASES, AND TEMPERATURE CHARTS FROM THE BUNDABERG GENERAL HOSPITAL.

Since the response of the sick children to treatment of various kinds might possibly have some bearing on the problem of diagnosis, we have summarized it briefly here. Cases 2, 8, 9 and 10 which presented no symptoms did not receive any immediate treatment.

Diphtheria antitoxin, 4,000 units intramuscularly, was given in cases 3, 4, 5, 12, 14, 15, 16 and 19 which died, and in cases 6, 7, and 13 which recovered. No antitoxin was administered in cases 1, 11, 18 and 20 which died and in cases 17 and 21 which recovered. Had the symptoms been due to the injection of free diphtheria toxin, much larger doses administered intravenously would have been indicated, and it is doubtful whether at the stage at which the treatment was given even this would have saved any lives. Dr. Hains, however, was concerned only with the possibility that a lack of balance between toxin and antitoxin in the immunizing injections might be a contributory cause of the illnesses. He was of the opinion that the administration of antitoxin had no influence whatever on the outcome.

Adrenaline in doses of 2 and 3 minims of 1 in 1,000 was given in a number of the cases. Apart from a temporary response to a dose of 5 minims obtained by Dr. McKeon in Case 19, the administration of adrenaline does not appear to have had any favorable result.

Atropine in doses from 1/150 to 1/200 grain with or without morphine 1/24 grain was given in most of the cases. There is no clear evidence that this treatment was of any value.

Oxygen was administered in most of the fatal cases and appeared to have but little effect on the cyanosis.

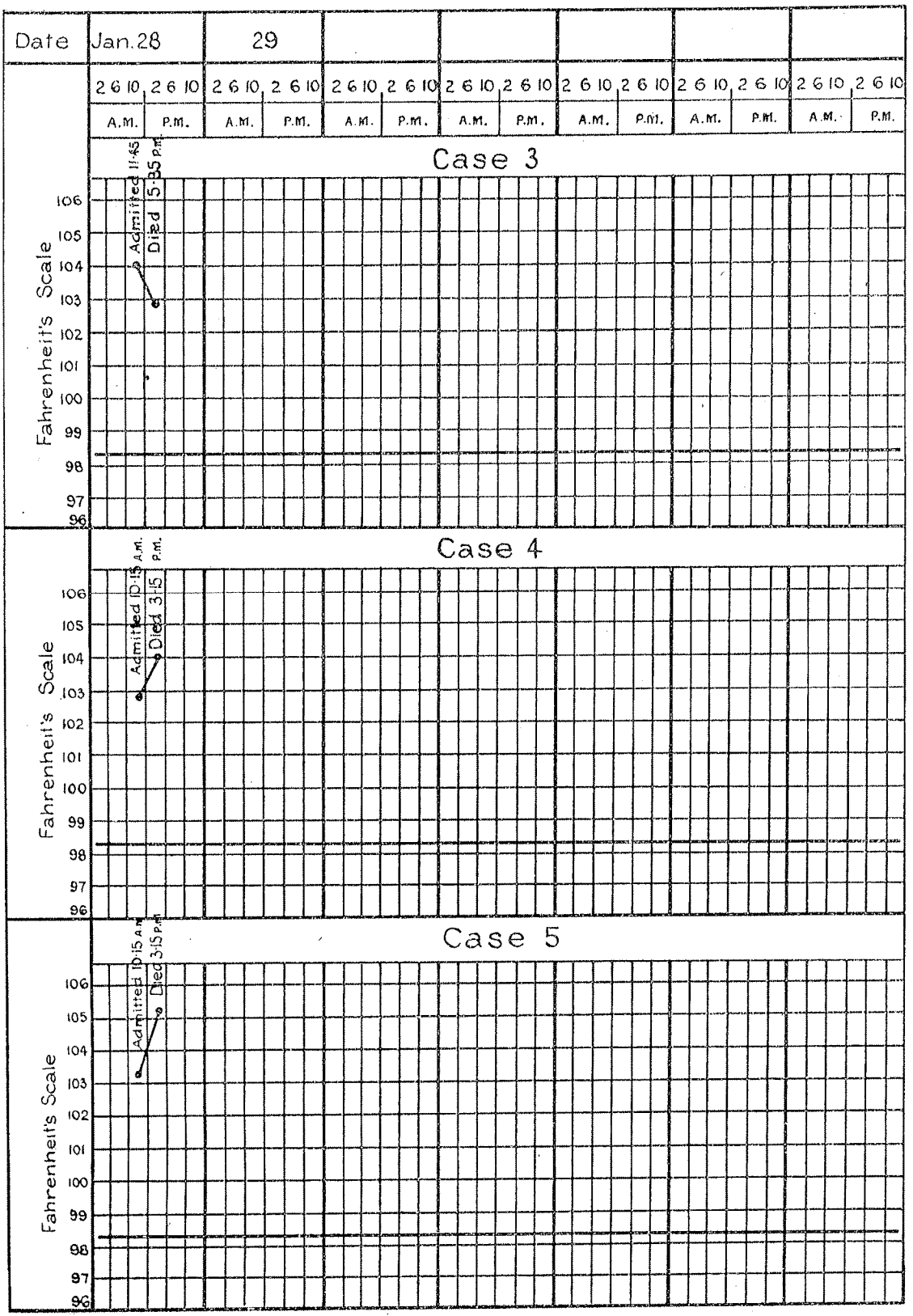
Digitalis was administered in a number of the cases and in Case 15, Dr. Hains thought it caused some temporary improvement. On the whole it was without effect.

The general treatment in most cases was the application of cold compresses to the head, sips of water to drink and frequent sponges, bowel washouts, enemata and purgatives. These measures appear to have been of value in the less seriously ill cases.

In Case 16 an attempt was made to control the convulsions with chloroform. This was without avail and was not persisted in.

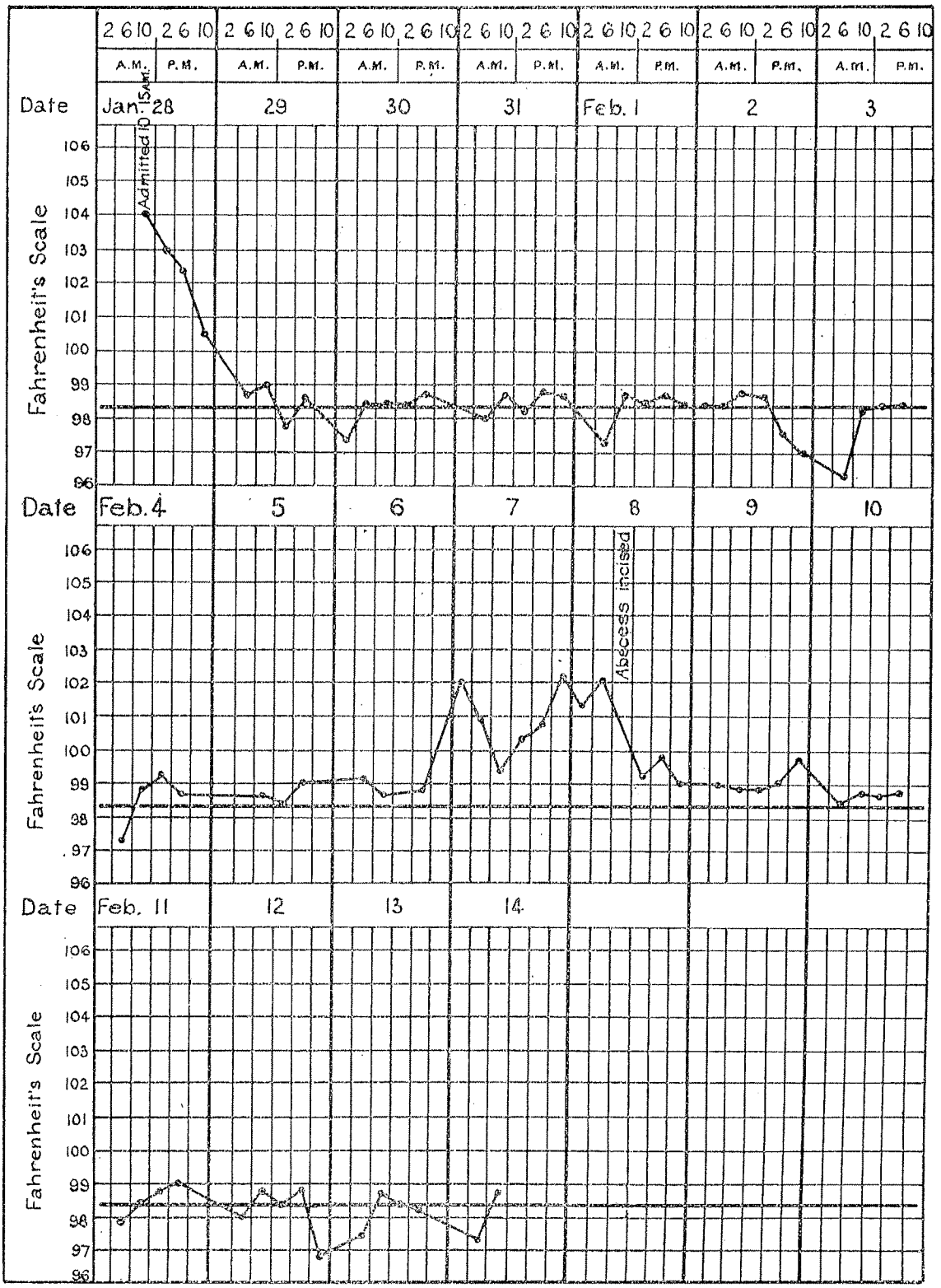
Temperature Charts

Cases 3, 4, 5



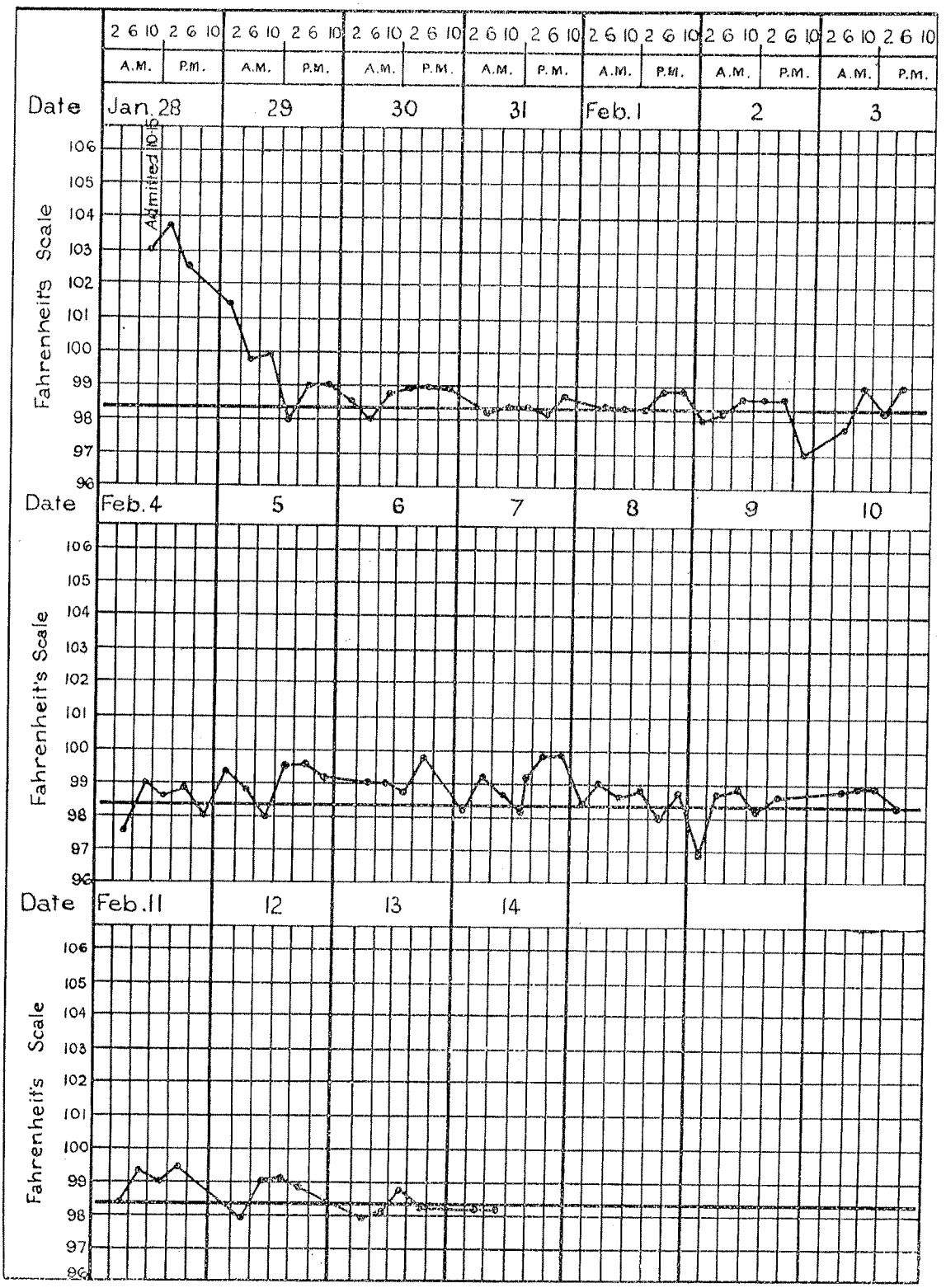
Temperature Chart

Case 6



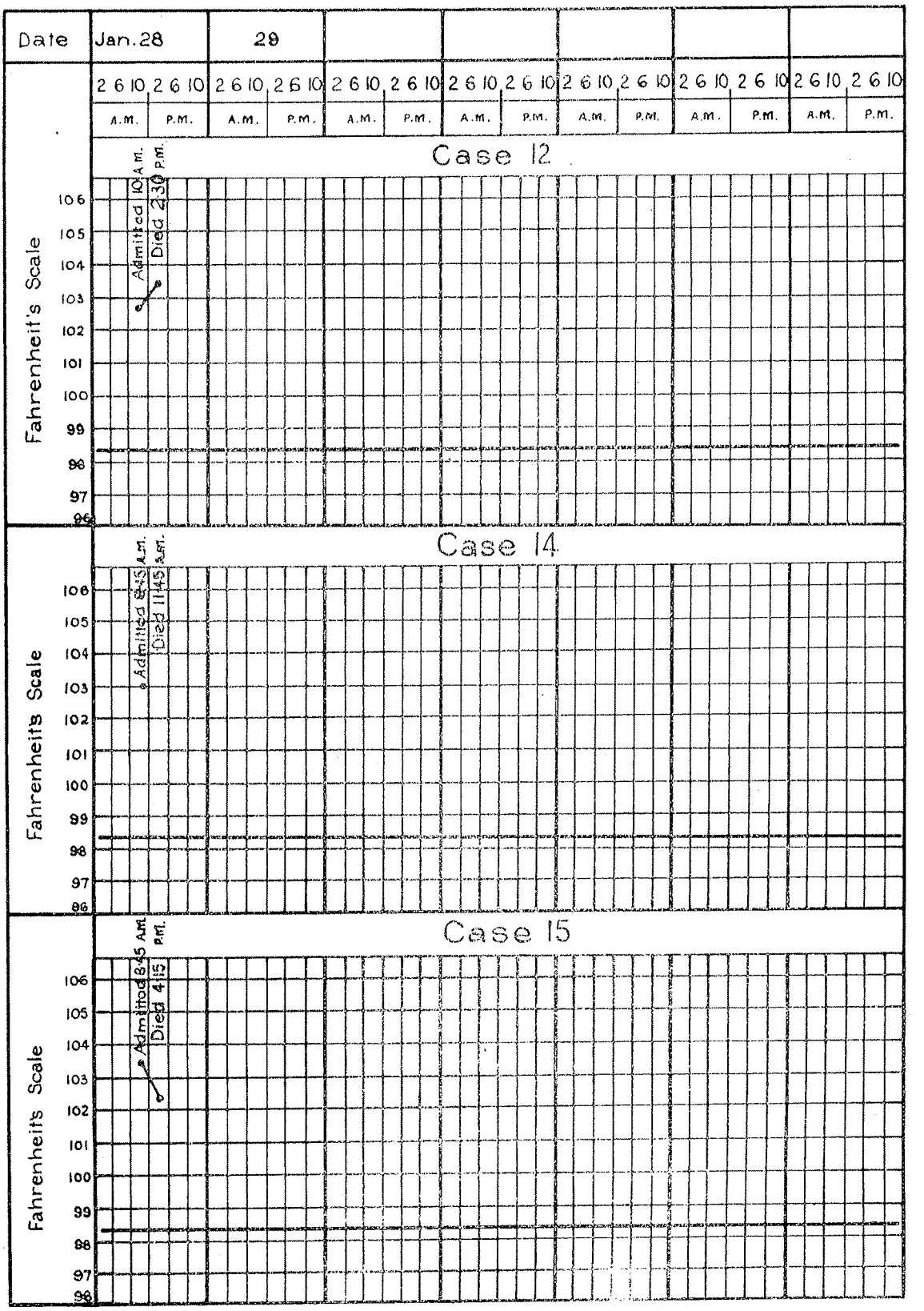
Temperature Chart

Case 7



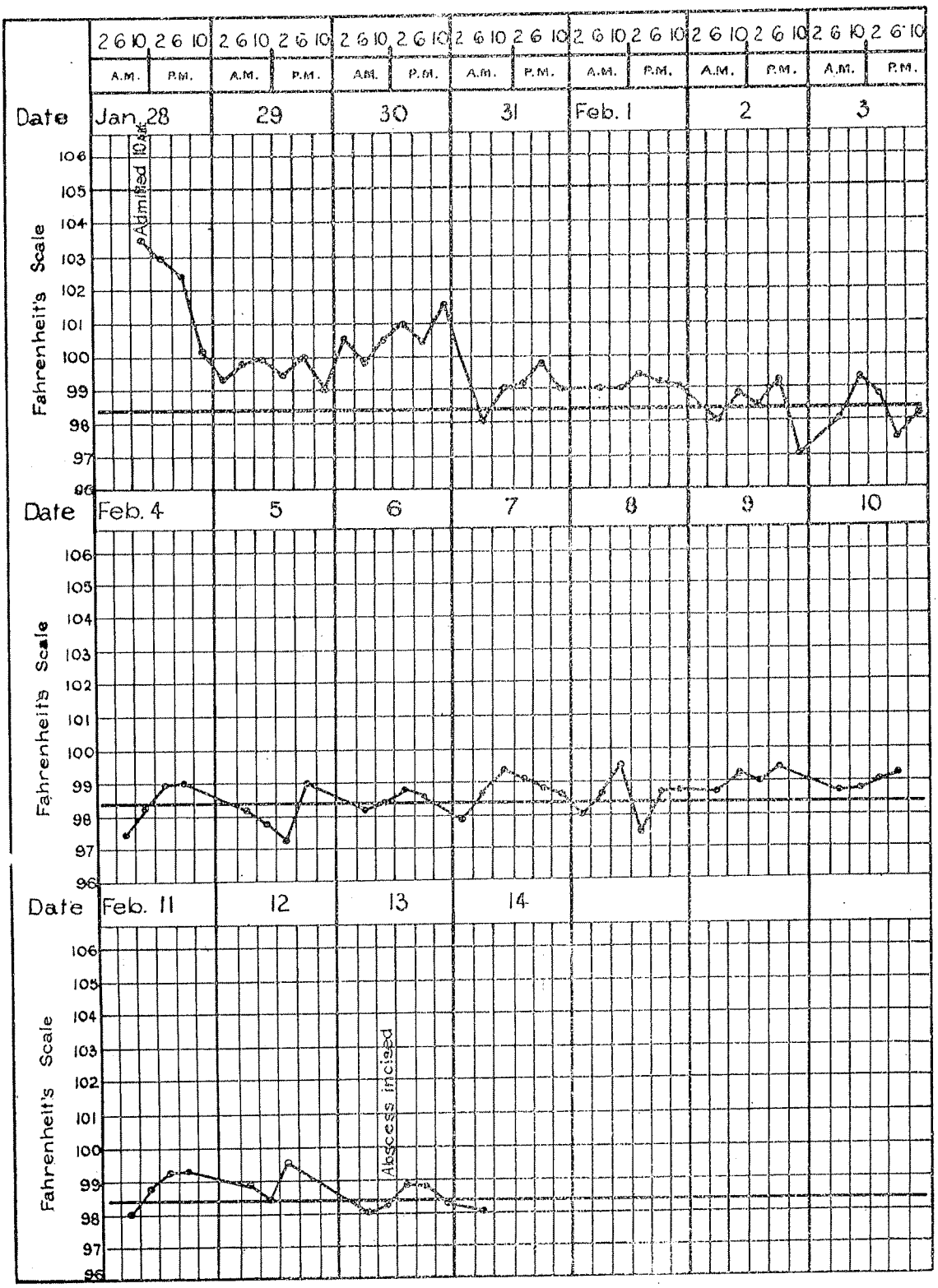
Temperature Charts

Cases 12, 14, 15



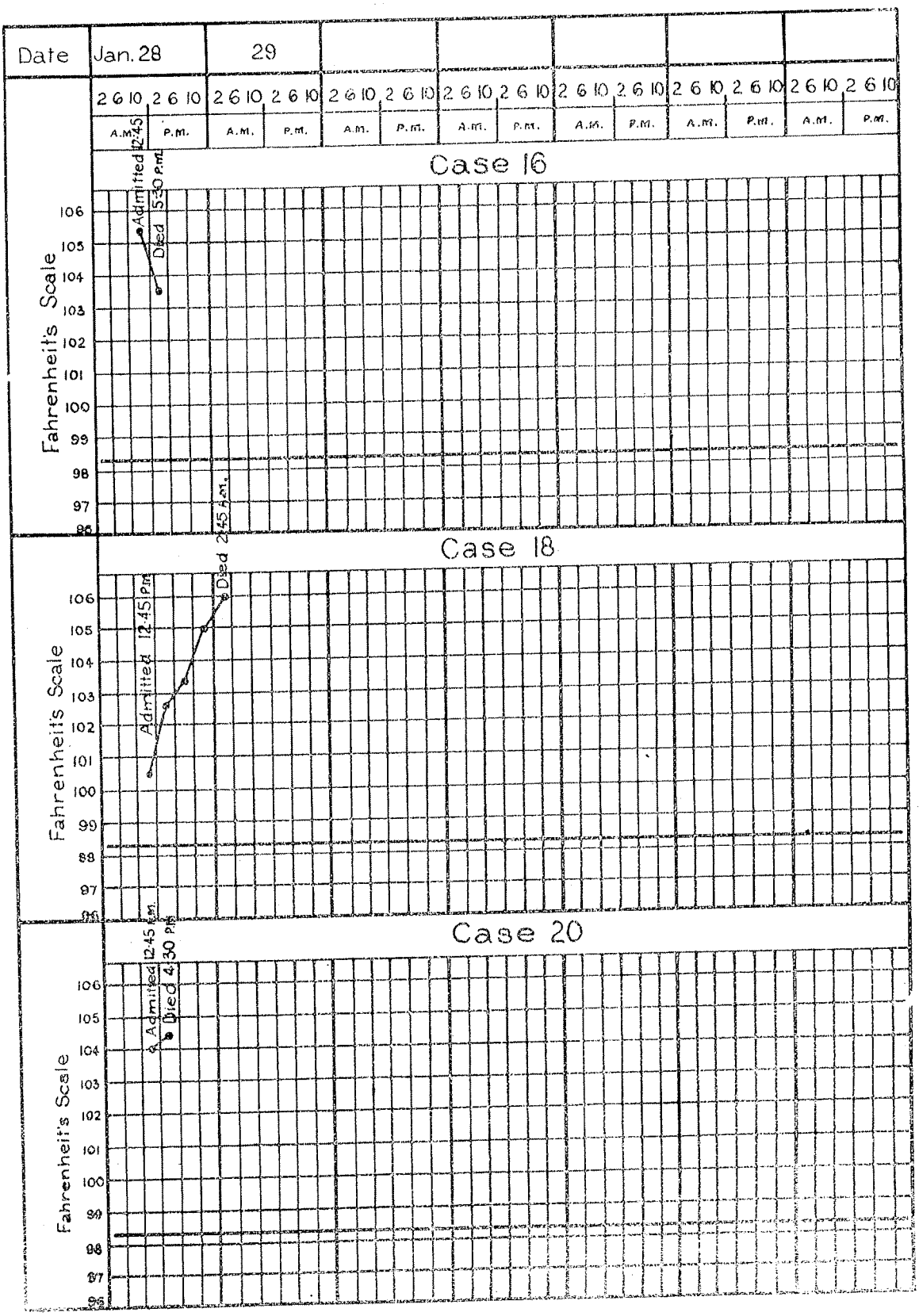
Temperature Chart

Case 13



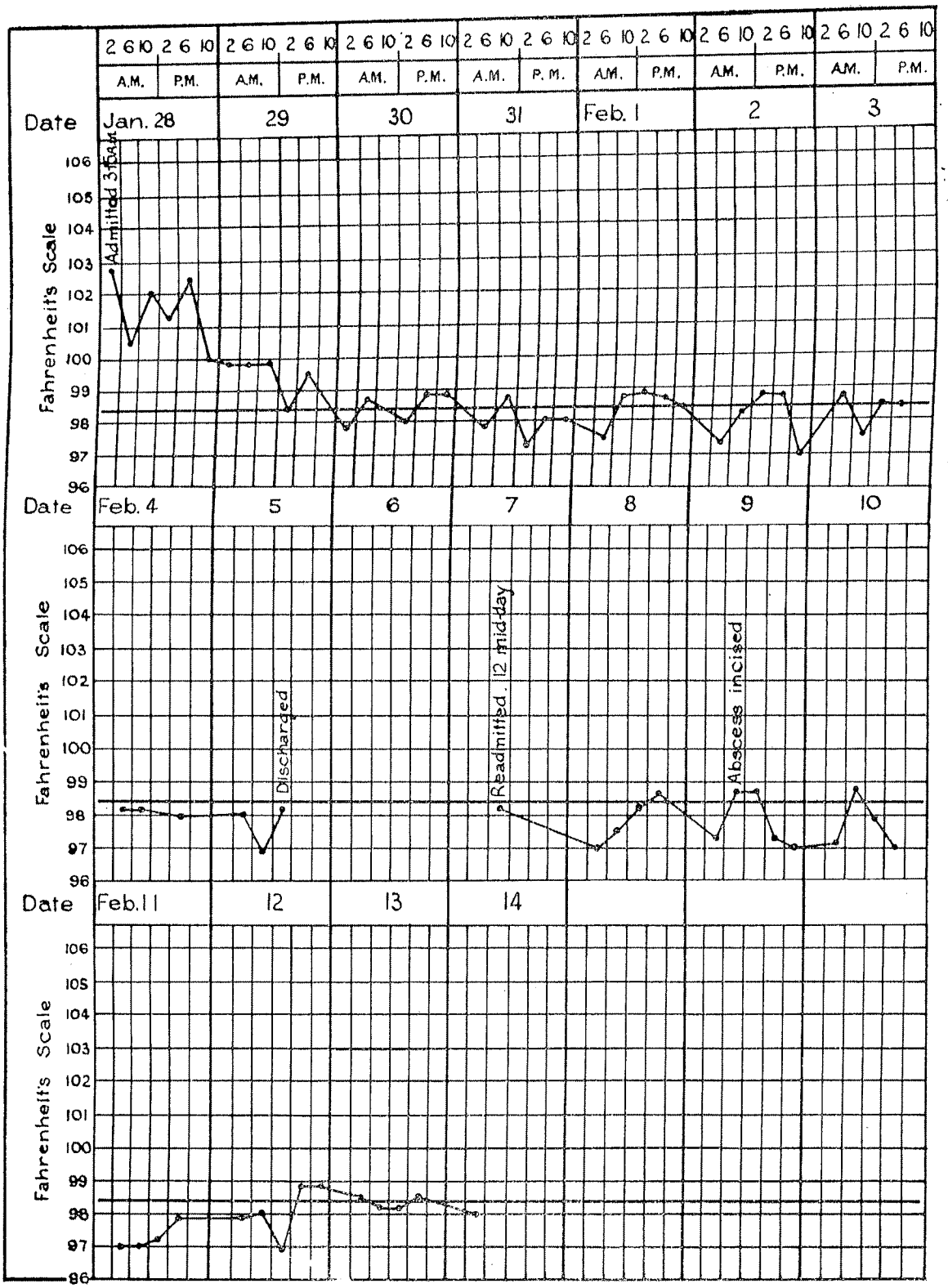
Temperature Charts

Cases 16, 18, 20



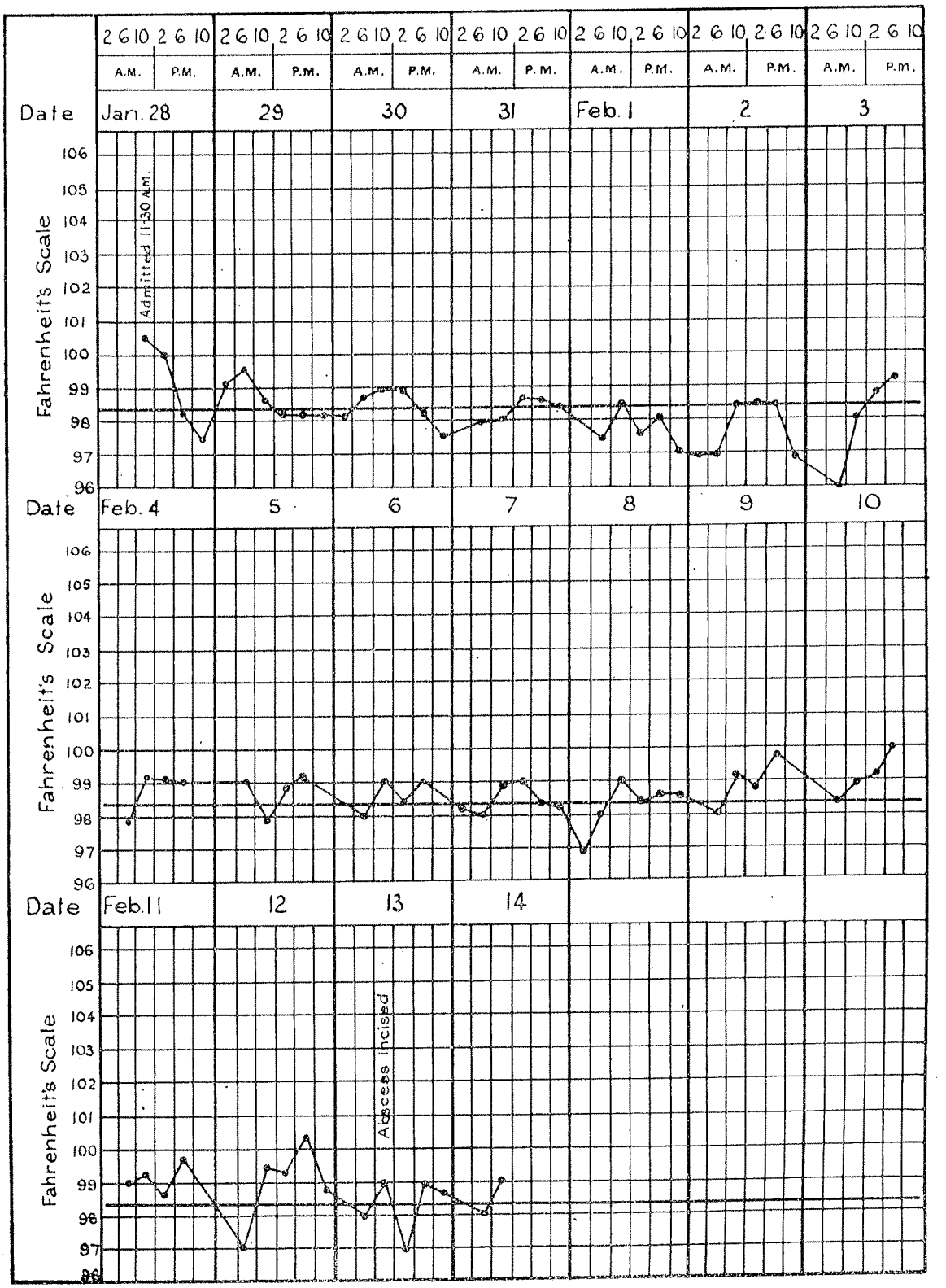
Temperature Chart

Case 17



Temperature Chart

Case 21



APPENDIX 9.

DETAILED NARRATIVE OF EVENTS IN RELATION TO THE REQUEST OF THE MAYOR OF BUNDABERG FOR A PATHOLOGIST.

Action taken at Bundaberg to procure a Pathologist.—Dr. E. T. K. Schmidt, during the morning of 28th January, 1928, notified the Senior Sergeant of Police at Bundaberg that he was not prepared to give a death certificate in the case of W.F., who had been brought to his surgery, already dead. An autopsy was conducted on the body of this child at 3 p.m. At about this time Dr. I. C. Hains, the Medical Superintendent of the Bundaberg General Hospital, where several deaths had already occurred, telephoned to the Senior Sergeant of Police at Bundaberg and intimated that he was not prepared to give certificates of death. Subsequently, the Sergeant telephoned the Medical Superintendent that his instructions from the Inspector of Police, Maryborough, were that post-mortem examinations were to be conducted by the Government Medical Officer (Dr. E. T. K. Schmidt). The Medical Superintendent then communicated with the Chairman of the Hospital Board and the Mayor of Bundaberg, and with their concurrence an urgent telegram was despatched at 5 p.m. to the Home Secretary, Brisbane, informing him of the disaster.

At about 6 p.m. the Mayor attempted to get in touch with the Home Secretary by telephone but was unable to do so. He then communicated with the Inspector of Police, Brisbane, and asked that the Home Secretary should be requested to send a pathologist by special train that evening to help the Government Medical Officer.

Subsequently, the police stated that they had been unable to locate the Home Secretary, but had communicated with the Under-Secretary of the Home Department, Mr. Gall, who was taking up the matter with Dr. J. S. C. Elkington, Director, Division of Tropical Hygiene, Commonwealth Department of Health, Brisbane.

On Sunday morning, 29th January, at 10 o'clock, the Mayor had a telephone conversation with the Home Secretary at his residence. The Mayor suggested the advisability of sending a competent pathologist by aeroplane. The Home Secretary said it was a Commonwealth matter, but that he would do everything possible. He later telephoned that Dr. Elkington, of the Commonwealth Department of Health, was arranging for Dr. G. A. Murray, a Quarantine Officer of the Commonwealth Department of Health, Brisbane, to leave for Bundaberg by the train on Sunday, the 29th January, and that Dr. R. E. Richards, of the Commonwealth Health Laboratory, Rockhampton, was also coming to Bundaberg.

State Action taken at Brisbane, re Pathologist.—At 6 a.m. on Sunday morning, the 29th January, the Home Secretary had a conversation with Dr. J. I. Moore, Commissioner of Public Health, Queensland, and inquired whether he had received any intimation relative to the fatalities at Bundaberg. He replied that he had and that he had already been in communication with Dr. Elkington, and suggested that, as the matter was a Commonwealth concern, Dr. Elkington should make all inquiries. At 10 a.m. the Home Secretary spoke on the telephone to the Mayor of Bundaberg, who requested that a pathologist should be sent by special train or aeroplane to Bundaberg. The reply of the Home Secretary was to the effect that the matter was one for experts, that nothing should be done at present, but that he expected to hear any moment from Dr. Moore, who was in consultation with Dr. Elkington. Dr. Moore subsequently informed the Home Secretary that Dr. Elkington had decided to go to Bundaberg. Dr. Moore was then instructed by the Home Secretary to place the full resources of the State Health Department at Dr. Elkington's disposal. Dr. Moore advised the Home Secretary that in his opinion the services of a pathologist were unnecessary. Later in the day Dr. Moore received advice from Dr. Elkington that he was unable to proceed to Bundaberg, but that Dr. G. A. Murray and Dr. Richards were going.

Commonwealth Action at Brisbane.—At 8.20 p.m. on Saturday, 28th January, 1928, Dr. J. S. C. Elkington, Director, Division of Tropical Hygiene, Commonwealth Department of Health, Brisbane, Queensland, received a telephone message from Dr. G. A. Murray, who had just been informed by Dr. Moore, giving him the first intimation of the deaths at Bundaberg. On receiving this information Dr. Elkington telephoned to Dr. J. I. Moore, State Commissioner of Public Health, Brisbane, who confirmed Dr. Murray's message stating that eleven (11) deaths had occurred. Dr. Elkington stated he would get in touch with Dr. Thomson at Bundaberg and advise Dr. Moore later what action had been decided upon. After obtaining an outline of the situation from Dr. Thomson, Dr. Elkington advised the Director-General of Health, Commonwealth Department of Health, Melbourne, of the fatalities, and telephoned Dr. Moore that he would send an officer to Bundaberg by the first available train. This officer (Dr. G. A. Murray) was despatched by Dr. Elkington only to investigate the situation from the administrative aspect. Dr. Moore stated that the Mayor of Bundaberg had asked for a pathologist to be sent, but Dr. Moore stated that he did not see the necessity for such action. Dr. Elkington refrained from commenting on Dr. Moore's view as he (Dr. Elkington) considered the situation a " domestic " matter of health.

APPENDIX 10.

DR. RICHARDS REPORTS ON THE AUTOPSY OF M.Br.

COMMONWEALTH OF AUSTRALIA.

DEPARTMENT OF HEALTH.

Commonwealth Health Laboratory,
Rockhampton, 3rd February, 1928.

28/64.

The Director, Division of Tropical Hygiene, Brisbane.

DEAR SIR,

The following are the details of the post-mortem examination performed by Drs. Schmidt and McKeon upon M. Br., aged two years and six months, 56 hours after death.

There was a marked bluish blotchiness of the skin on the limbs and trunk, being apparently due to intracutaneous haemorrhages.

This feature, I was told, was characteristic of all the other cases and was attributed to the injections of adrenaline or antitoxic serum, 4,000 units, given before death.

The chest wall was opened and the pericardium appeared normal containing a little fluid, which was cultured on serum agar plates.

The heart was markedly contracted, this was opened and some of the fluid blood cultured on serum agar plates.

The lungs were then examined and appeared to be normal.

The abdomen was then opened and the peritoneum was well spread out, pale and not injected. The stomach and intestines were pale both inside and out, showing no injection.

The spleen, liver, kidney and pancreas were examined in turn and did not appear to be different from normal.

One feature was our inability to find any well-defined suprarenal gland tissue on the upper pole of the kidney in the fat. We, however, removed the whole upper pole with the fat in the hopes of finding adrenal tissue therein later.

The brain and meninges were then exposed, both were pale with no marked injection.

Upon asking the operators if the post-mortem examination showed the same changes as the ones on the fresher corpses, they said it was so, except in the cases of brain, which showed marked injection with small petechiae in the white matter and an ante-mortem clot in the superior and inferior venae cavae.

This was also corrobated by Dr. Hains.

Cultures of the meningeal fluid, liver and spleen were taken on blood agar slopes.

Pieces of heart, lung, liver, spleen, pancreas, meninges and brain were taken for microscopic examination.

Yours faithfully,

(Sgd.) R. E. RICHARDS.

COMMONWEALTH OF AUSTRALIA.

DEPARTMENT OF HEALTH.

Commonwealth Health Laboratory,
Rockhampton, 4th February, 1928.

28/69.
Memorandum.

The following is a report on the Microscopical Examination of organs removed from M. Br., post-mortem, Monday, 30th January.

Liver.

The lobules appear well defined.

The portal canals appear normal. Some of the intralobular veins seem more distended than normal.

The liver cells do not show any definite change. There may be a little more shrinkage than normal making the intercellular lymph spaces larger, probably a post-mortem change.

The capsule seems unchanged.

Heart Muscle.

The muscle fibres show well-stained nuclei, and are definitely striated.

There seems to be more fragmentation than normal, but this is probably a post-mortem change.

There are no signs of degeneration in the muscle cells themselves.

Arteries and fibrous tissue seem normal.

Spleen.

Microscopic examination of the spleen does not reveal any changes from the normal organ.

Lung.

The epithelium lining the alveoli appears normal. The alveoli themselves do not show abnormal contents in the way of any exudation.

The blood vessels do not show abnormal content of blood cells.

Kidney.

The glomeruli appear normal. There is no distension of the Bowman's capsule caused by any excess exudation from the capillaries, nor is there excess of lymphocytes.

The epithelium lining the convoluted tubules in the vicinity of the glomeruli shows granular changes and loss of staining power.

I am inclined to think this has been caused by the " toxins " as the tubules lower down show well-defined and deeply-stained epithelium.

The blood vessels appear normal.

Pancreas.

In parts of the glands, the cells show a degenerative change, i.e., lack of definition and staining power.

In other parts the cells stain fairly well, but there is considerable shrinkage leaving the fibrous tissue framework very well marked.

There is no marked inflammatory change in the way of increased blood vessels and increase in leucocytes.

Brain.

The nervous tissue appears normal.

The blood vessels and capillaries do not show any engorgement.

Further than this I am unable to comment on brain tissue.

From these findings it will be seen that the only changes were in the kidney and pancreas. This is only a toxic spoiling characteristic of any toxaemia.

I failed to find any adrenal tissue in the fat on the upper poles of the kidneys.

This may be due to lack of anatomical knowledge on the part of Dr. McKeon and myself, but we spent quite a considerable time looking for the adrenal glands and finally decided they must be in the fat.

I am forwarding a duplicate set of these slides to you.

I would be pleased to hear the report on them from some other pathologist.

(Sgd.) R. E. RICHARDS,
Medical Officer in Charge.

The Director,
Division of Tropical Hygiene,
Box 425 F., Brisbane, Queensland.

APPENDIX II.

POST-MORTEM EXAMINATION OF THE TISSUES RETAINED FROM THE AUTOPSIES AT BUNDABERG.

Tissues and organs were retained from the autopsies on four bodies, those of J.P., W.F., T.R. and M.Br. The autopsies were performed at the following periods after death:—At 7½ hours, 7 hours, 8¼ hours and 52½ hours respectively.

The tissues examined by the Commission were—

Tissue.	Case 12, J.P.	Case 1, W.F.	Case 18, T.R.	Case 16, M.Br.
Heart	..	Whole	Whole	Portion
Lungs	..	Left	Both	Portion
Thymus	..	Whole	Whole	..
Stomach	..	Whole	Whole	..
Intestines	..	..	Whole	..
Liver	Portion	Whole	Whole	Portion
Pancreas	..	..	Half	Portion
Spleen	Whole	Whole	Whole	Portion
Kidneys	One	Both	Both	Portion
Suprarenals	..	Right	Right	..
			One-third of left	
Brain	..	..	Whole	Portion
Dura Mater	..	..	Portion	Portion
Lymphatic Glands	..	Abdominal and Bronchial	Abdominal and Bronchial	..
Clot from Vena Cava	One Specimen	..	..	..

In addition the smears taken from organs at the autopsy on M.Br. (Case 16) by Dr. Richards were examined. These were marked Liver (? Lung) and Spleen. Films from his cultures from pericardial fluid and heart blood were also examined.

The weights of the organs where possible were determined on arrival in Melbourne. The weights in grammes were as follows:—

——	J.P. Aged 5½ Years.	W.F. Aged 2¼ Years.	T.R. Aged 5½ Years.
Heart	..	50 (58·9)	70 (85·5)
Lungs	..	115 (90)	380 (318)
Thymus	..	15·5 (15·6)	14·7 (15·6)
Liver	..	450 (470)	460 (580)
Spleen	26·5 (50)	56 (43)	59 (56·5)
Kidneys	36·5 (56)	L–33·2 R–29·5 (53)	L–36·6 R–36·2 (56)
Suprarenals	..	R–·84 (1·3)	R–·97 (1·375)

The figures given in brackets are the average weights of the organs for the age and sex (after Vierordt). The weights give little information, making due allowance for the variability of the organs, and for the fact that the method of preservation used causes great reduction in weight of an organ. Similar pieces of kidney and liver so treated lost 20 per cent. and 14 per cent. of their weight in a week. The spleen is possibly enlarged in W.F. and T.R. (Cases 1 and 18) and remarkably small in J.P. (Case 12). The kidneys are uniformly below what we should expect dehydration during preservation to account for. The high values for the lung are probably due to incomplete removal of fixing fluid. The bodies of the children were not weighed at autopsy and no accurate information is available to allow consideration of the relationship to body weight.

The changes which were found to be present in the organs of the children were so similar both macroscopically and microscopically that it will be convenient for the purposes of this report to give a general description and note individual variations where these occur.

One feature common to all the cases except M.Br. (Case 16) is that owing to the way in which the specimens had been preserved, the superficial tissues of the organs were brittle and discoloured to a variable depth, while satisfactory fixation was correspondingly limited. The scope for observation in certain directions was, therefore, restricted.

Liver.—Two of the organs were complete (W.F. and T.R.)—

	W.F. (case 1)	T.R. (case 18)
Weight	450 gm.	460 gm.
Greatest vertical measurement (near gall bladder)	12 cm.	10·5 cm.
Greatest transverse measurement (near neck of gall bladder)	14 cm.	15·5 cm.
Greatest anteroposterior measurement (near neck of gall bladder)	4·75 cm.	4·75 cm.

Viewed from the front both are roughly quadrangular and the lower margin of the right lobe is bluntly rounded in each case.

The peritoneum is thin, transparent and free from hæmorrhages or exudate. The fine mosaic of the underlying lobules is just visible through it. On section the organ has a uniform very pale buff color. The hepatic veins are somewhat distended and filled with clot and a similar distension of less degree is seen in the portal veins. The central lobular veins are just visible and not obviously distended. No excess of fibrous tissue is present and no evidence found of hæmorrhage or focal lesion of any kind. No changes are observed in the structures in the porta hepatis or ligaments. In the case of W.F. (Case 1) though no gas formation had occurred, the central portion of the liver when first sectioned was a brown spongy mush.

Microscopic.—The capsule is not thickened but is finely wrinkled. The peritoneal endothelium appears to be intact. There is some indeterminate debris on the surface which, however, is not exudate and there is no suggestion of peritonitis. There are some dilated veins distinguishable in the pigmented shrunken tissue.

Beneath this surface alteration the usual architectural characters of the liver are maintained. The sinusoids are uniformly dilated throughout the liver lobules. This is not a shrinkage effect (though varying degrees of this are seen), for blood fills the sinusoids. The capillaries in the portal tracts are similarly dilated. The veins both central and portal are likewise fully charged with blood. The arterioles are not affected in this manner and some of them have a contracted appearance. The distension of the sinusoids is not especially marked round the central veins, nor is the condition of the cells there in any way different from that of those near the portal tracts. The contour of the liver cells in spite of the separation of the columns gives them a slightly turgid appearance. The cytoplasm is granular, and shows no excess or special localization of fatty accumulation. Chromatolysis is advanced in the nuclei of many of the cells, though the majority retain their staining characters well.

No swelling or other change is detected in the vascular endothelium. There is no thickening of the walls of the veins.

What appear to be widely dilated lymphatics with finely granular contents are seen alongside the large arteries and veins in the portal tracts. A notable feature is their content of fairly numerous polymorphs, which is the more remarkable as the impression obtained from an examination of the blood in the vessels does not suggest a leucocytosis of this kind.

It is also noted that in the connective tissue of many of the portal tracts and sometimes extending along between the lobules, there is a general slight but definite excess of cells, mostly mononuclear or lymphocytic, but a fair proportion of them of the polymorphonuclear variety. At no point does this cellular increase amount to a focal lesion. No abnormal biliary or other accumulations of pigment are noticeable. No pathological features are seen in the Küpffer cells. The bile ducts also show no change, the epithelium being in good condition. Their lumina contain granular material. No focal lesions or haemorrhages are seen.

In the sections from W.F. (Case 1) the tissue is badly altered by post-mortem change. More vacuolation is seen in the sections from W.F. and T.R. (Cases 1 and 18). In the latter case it is present in some excess, but it is doubtful if it is of any pathological significance. The same slight but definite cellular infiltration is present in the other cases, but in none of them are the polymorphs in such high proportion.

Gall Bladder.—The organ was examined in W.F. and T.R. (Cases 1 and 18) and shows no obvious lesions on the peritoneal or mucous surfaces. There is no distension or thickening of the wall. The bile had been evacuated before the organ was examined.

Microscopic.—The gall bladder is severely affected by post-mortem changes. The epithelium has mostly disappeared. Apart from much vascular dilatation, no significant changes are seen in the walls.

Spleen.—Three of the organs were complete (J.P., W.F., and T.R.).

	Weight.	Dimensions.
J.P. (Case 12)	26·5	7·2 x 4·7 x 1·4 cm.
W.F. (Case 1)	56	7·5 x 4·75 x 2·9cm.
T.R. (Case 18)	59	7·1 x 4·5 x 2·5 cm.

The peritoneal surface is without lesion, and nothing abnormal is noted about the arteries at the hilum. The vein is wide and full of red clot. The surface exposed on section show as greyish brown colour dotted prominently throughout with paler buff circular areas of approximately ·5 c.cm. diameter, the malpighian bodies. The surface is somewhat granular and the pulp looks congested and has minute black dots scattered through it. A number of veins plugged with red clot show up on section. There is no gross change visible in the arteries nor special prominence of fibrous tissue.

Microscopic.—The changes most obvious on general inspection are sinus engorgement and focal lesions in nearly every malpighian body.

The capsule presents no unusual features.

The larger vessels in the trabeculae are all somewhat engorged.

The malpighian bodies are all much enlarged with a peripheral lymphocytic concentration and a central area of poor definition in which much cellular degeneration is evident. In a typical focus the periphery of the follicle is densely packed with lymphocytes. Eccentrically placed is usually an arteriole which shows no abnormal features beyond in some cases very slight swelling or fusion of the inner layers giving a hyaline appearance. Several rather dilated capillaries are sometimes to be seen. The centre and main bulk of the follicle consist of more loosely arranged degenerating cellular material in which the more numerous and best preserved nuclei recognizably belong to cells of the reticular type. The remaining nuclear material is pyknotic and derived from lymphocytes or reticular cells.

The sinuses are much engorged, their contents being chiefly red blood corpuscles with fairly numerous leucocytes of lymphocytic and larger mononuclear types. There is no swelling or other readily recognizable change in the endothelium. The pulp between the sinuses shows mostly a very loosely arranged reticulum in the meshes of which in the more congested areas erythrocytes predominate, while in other areas there are more leucocytes mainly of mononuclear type. The amount and distribution of pigment does not indicate either excessive haemolysis or abnormal phagocytic activity. Occasional degenerating cells and phagocytosis can be found in the pulp.

This description applies to the appearances seen in the other cases except that the central degenerative changes in the malpighian bodies are even more marked in the case of T.R. (Case 18), while the lymphoid tissue is less dense, more diffused and poorly defined. In W.F. and M.Br. (Cases 1 and 16) the degenerative changes are not so marked, though in M.Br. affected reticulum is seen beyond the zone of lymphocytes. The hyaline appearance of the inner layers of the arterial walls is very slight and inconstant.

Kidneys.—

Both organs from two cases and one from a third were retained.

		Weight.	Dimensions.
W.F.	L.	33·2 gm. ..	8·1 x 3·4 x 2·1 cm.
	R.	29·5 gm. ..	6·8 x 3 x 2 cm.
T.R.	L.	36·6 gm. ..	6·75 x 3·6 x 2·4 cm.
	R.	36·2 gm. ..	6·7 x 3·6 x 2·5 cm.
J.P.	L.	36·5 gm. ..	7 x 3·75 x 2 cm.

Apart from minor unimportant anatomical differences, the same description will apply equally well to all the organs examined.

The capsule is not thickened and strips easily and cleanly, leaving a surface not noticeably congested and on which no hæmorrhages or other focal lesion can be detected. On some areas the fine mottling appears to define the surface projection of the cortical structures on the capsule.

Section gives no indication that the kidney tissues were under any undue tension. The cortex has a depth of about ·4 cm., and is quite regular in structure, There is some slight congestion visible directly under the capsule, occasionally extending for some distance between the cortical columns or medullary rays, which have a slightly swollen appearance when examined with a hand lens. The glomeruli are not specially prominent. The pyramids are darker in colour than the cortex, and many of them show minute dark points or streaks indicating some slight congestion. This is especially noticeable in the intermediate zones. The papillae present no unusual features. No infarcts, abscesses or other gross indication of any focal lesion are seen.

The larger blood vessels are considerably engorged. The arterial walls are not prominent on section and have no gross appearance of disease. There are no abnormal appearances in the structures in the sinus renalis.

The engorgement of the larger vessels in the intermediate zone is particularly marked in the organs from W.F., T.R. and J.P. (Cases 1, 18 and 12) and widely dilated vessels are not uncommon in the cortex. In the last at autopsy there was some suspicion that an infarked in present at the lower pole of one of the kidneys. If this be the organ retained, the infarction cannot now be distinguished.

Microscopic.—The capsule is thin and beneath it are seen somewhat engorged veins. The architecture of the cortex is not grossly disturbed. The glomeruli stand out in staining contrast to the surrounding tubular tissue. The capsular spaces are fairly wide, and the glomerular capillaries are uniformly but not excessively engorged. No thickening of Bowman's capsule is seen in any part, no swelling of its epithelium, nor are adhesions or hæmorrhages to be detected. The convoluted tubules show a uniformly marked swelling of the epithelium (often almost obliterating the tubular lumen) and granularity of cytoplasm, while many nuclei stain very faintly. No vacuolation of the cytoplasm is present. The margins of the cells are in most cases fairly well defined, a little debris only being found in any of the lumina, while casts and blood are not found at all. The loops of Henle and collecting tubules show better nuclear preservation, though especially in the deeper parts of the section the cellular preservation is not satisfactory.

Throughout the section the intertubular capillaries, both in cortex and medulla, but perhaps more especially in the intermediate zone, show a general engorgement with local exaggerations. Red blood corpuscles everywhere stain badly. The larger vessels, both arteries and veins, are distended with blood, it being exceptional to find a collapsed vein. No endothelial change is to be detected in the capillaries. No hæmorrhages are present in any part of the sections examined nor are any focal accumulation of cells or areas of necrosis or other focal lesions observed.

In T.R. (Case 18), the engorgement in the capsule and immediately subjacent tissue is rather more marked. Most of the glomeruli are rather more congested and in contrast to the description given, fill their capsules and sometimes show an irregular contact that suggests adhesions. The glomeruli throughout give no other structural indication of past or present lesion. In M.Br. (Case 16) the post-mortem deterioration is much more advanced than in any of the other cases. In M.Br. and W.F. (Cases 16 and 1) the congestion is more patchy in distribution.

Suprarenals.—The right suprarenal is available from W.F. and T.R. (Cases 1 and 18) and about half of the left from T.R. (Case 18).

	W.F. (case 1)		T.R. (case 18)
Weight	·84 gm.	..	·97 gm.
Dimensions	3 x 1 ·4 x ·5 cm.	..	3 ·1 x 1 ·5 x ·35 cm.
Thickness	·15 cm.	..	R— ·17 cm. L— ·17 cm.

Both right suprarenals were found attached to liver and the left to the upper pole of the left kidney.

Macroscopic.—Except that they had been slightly deformed by pressure, there is no departure from the usual form to record.

On section the medulla shows merely as the dividing line between the layers of the cortex, which is of a pale yellow colour with regular striation visible on close examination. There is no trace of hæmorrhage or congestion apart from the fact that there was a visible clot in the central vein of the right suprarenal of T.R. (Case 18). The appearance of this organ in all three specimens is perfectly normal.

Microscopic.—The capsule shows no congestion. The suprarenal tissue is much affected by post-mortem change. The general structure, however, is seen to maintain the normal relationship of the zones in accordance with the age of the subjects. In the glomerulosa (a narrow zone) and the fasciculata vacuolation presumably corresponds to the removal of lipins, which are uniformly distributed and more especially developed in the fasciculata (normal). The reticularis and medulla are less well defined. The cortex shows no necrosis. There are no areas of leucocytic infiltration. There is no hæmorrhage. There is perhaps a very slight general capillary dilatation and some engorgement of the larger blood vessels. The medullary cells show no hyaline change.

Pancreas.—About half the organ of T.R. (Case 18) and a small portion of that of M.Br. (Case 16) were kept.

Macroscopic.—The organ is soft and the lobules of pancreatic tissue of a uniform pinkish tint (greyish white in the specimen from M.Br.). The connective tissue does not appear to be excessive and the ducts show no abnormal features. No congestion is seen macroscopically and no hæmorrhages or other macroscopic lesions are discernible. The splenic vessels in relation to it show no changes of note in the preserved state.

Microscopic.—The tissue is with difficulty recognizable, so advanced is the post-mortem change. Some of the larger vessels appear to be congested. No focal lesions or hæmorrhages can be discovered.

Stomach.—Two organs were examined—W.F. and T.R. (Cases 1 and 18).

The length along the lesser curvature is approximately 5 cm. The stomach is neither contracted nor dilated. There are a few congested veins radiating for a short distance over the anterior surface from both curvatures. Otherwise the peritoneum is uniformly pale grey. No peritonitis or sub-peritoneal hæmorrhages are present. The mucous membrane apart from the usual folds is fairly smooth and on close examination intact, no ulcerations, hæmorrhages or or other focal lesions being observed. It has a pinkish tinge in some areas, but there is no congestion. The lower end of the oesophagus and the pylorus are similarly free from abnormality. The fundus of the stomach of W.F. (Case 1) is slightly distended and thinned out. A few dilated veins are visible in this part on transillumination.

Microscopic.—This organ has suffered much from post-mortem changes.

The mucous membrane, however, appears to be quite regular in structure, though many of the more superficial cells have disappeared. There is no swelling or congestion in the stroma. No notable reaction in the lymphoid tissue is found. Scattered through the mucous membrane in small numbers are seen lymphocytes and occasionally a few polymorphs. There is some slight vascular dilation in the submucous tissue, but no changes are seen in the muscular or peritoneal coats.

Intestines—These were only examined in one case, T.R. (Case 18).

Macroscopic.—The Small Intestine.—The duodenum is slightly distended. The jejunum for the first 3 feet is considerably distended, probably by gas. Thereafter the lumen of the bowel is for the most part small and somewhat contracted with a few short lengths of moderate dilatation. The contracted condition of the bowel is more marked in the ileum than in the jejunum. The contents are scanty and pultaceous and, except in a few lengths of bowel, bile stained.

The Peritoneum.—Much of this, from unsatisfactory preservation, is of no value for examination, but where observation can be made, is seen to be smooth, shiny and transparent, with no thickenings, adhesions or exudate. There is some very slight fine sub-peritoneal vascular congestion in the first part of the duodenum and a little similar congestion in the first part of the jejunum chiefly at the mesenteric attachment. Apart from this insignificant engorgement, there is no congestion to be seen, the surface generally being pale.

The mucous membrane reveals no gross lesions; no ulcers, or areas of congestion, inflammation, necrosis or hæmorrhage being detected. Beyond the stretching out of the mucous membrane in dilated parts and increased rugosity in the contracted areas, no significant change is evident.

In all but the lowest portion of the ileum the lymphoid tissue is not noticeably swollen or obviously congested and no affection of the Peyer's patches is visible macroscopically. In the last foot and increasingly towards the ileocolic valve the lymphoid tissue is prominent, the follicles and Peyer's patches projecting from the surface. No necrotic foci or other gross changes are visible on inspection of the surface or on section of these lymphoid projections. A central depression is readily visible on the summit of each follicular mass. The mucous membrane of the last 2 inches of the ileum is very rugose and appears to be swollen. The ileum projects prominently into the cæcum.

Appendix.—Length 8 cm. The appendix was retrocaecal in position. It shows no swelling or congestion. A little fæcal accumulation was found in its lumen 2 cm. from the orifice.

Large Intestine.—The peritoneal surface, apart from some very slight venous congestion in the sigmoid region, is pale and presents no pathological features.

The cæcum is dilated, the ascending and transverse colons somewhat contracted. The descending and sigmoid colons are dilated except for a few very short lengths of bowel, which are firmly contracted.

The lymphoid tissue in the cæcum and throughout the large intestine is somewhat prominent. The mucous membrane is everywhere intact as far as can be determined by naked eye inspection, and there is no lesion discernible at or about the sites of contraction in the descending colon. Bile staining is evident in the cæcum and some of the pultaceous food material lower down in the bowel (sigmoid) is also tinged with bile. No mucus is seen in the preserved specimen. No formed fæces were found in any part of the bowel.

Microscopic.—Duodenum.—There is much disappearance of the surface cells of the mucous membrane and at the tips of the denuded villi the cells of the stroma show faded nuclei or none, and stain poorly. The epithelium in the deeper parts of the crypts is sometimes healthy looking, though shrunken away from its basement membrane, but in many places the cells have swollen, become vacuolated, or degenerated or have completely disappeared. The cells of Brunner's

glands also have a swollen appearance. There is little indication of congestion in the submucosa, and no alterations are seen in the muscular, sub-peritoneal or peritoneal layers. The abnormal appearances are probably in the main of post-mortem origin.

Jejunum.—In this portion of the bowel the epithelium is less altered, but much separation of strips of membrane is seen at the surface. The villi, however, show a good deal of capillary congestion and are somewhat oedematous. The vessels in the stroma of the valvulae conniventes are also engorged, and this vascular condition and some slight oedema are general throughout the submucosa. In addition to the usual leucocytes, there are also in the submucosa and of the villi a considerable number of polymorphs, which together with lymphocytes are also seen amongst the cells of the mucous membrane, in fair number in the lumina of some of the crypts, and in places associated with local disintegration of the mucous membrane. These cells are very few amongst the desquamated cells on the surface, which has not the appearance of an exudate. Nevertheless the picture is inflammatory in kind, though not of severe degree. The muscular tissue, subperitoneal and peritoneal coats show no appreciable alteration.

Ileum.—In the upper part of the ileum there is little or no congestion and the mucous membrane displays no special feature beyond the diappearance of many of the surface cells. Towards the ileocaecal junction, however, there is an increasing submucous congestion and much swelling of the lymphoid tissue. The lymphoid tissue of the Peyer's patches and agminated glands shows numerous dilated capillaries and is oedematous. The " germ centres " are large and of loose structure, and show similar features to the lymphoid tissue in the spleen, granularity, and lack of cytoplasmic definition, with poor affinity for stain, and nuclear degeneration and fragmentation. The villi in these areas are mostly denuded of epithelium. In the intervening parts similar loss of epithelium is seen and congestion and oedema of the stroma are present in varying degree. Polymorphs are rare. There is a mixture of amorphous granular material and degenerating cells on the surface. The congestion is present also in the vessels of the muscular coats, which, however, are otherwise not affected, while the peritoneal and subperitoneal tissues show no apparent change. No hæmorrhages are found.

Cæcum.—At the beginning of the large intestine the post-mortem changes obscure a picture which in the better preserved parts is seen to be essentially similar in character. The changes in the lymphoid tissue are the same. Some congestion, submucous and mucous, and a certain amount of oedema, together with desquamation of epithelial cells are common to all the sections examined.

Different portions examined differ in their preservation. Lower down in the large intestine submucous congestion and superficial desquamation is still seen, but in diminished degree.

Lungs.—From T.R. (Case 18), both lungs, from W.F. (Case 1) the left lung, and from M.Br. (Case 16) a portion of one lung were examined.

Macroscopic.—T.R. (Case 18)—combined weight, 380 gm.

W.F. (Case 1)—left lung, 115 gm.

The organs are much distorted from pressure.

No evidence of exudate or hæmorrhage is seen on the pleural surface. There are no pleural thickenings or remains of adhesions. The cut surface of the lung has a reddish brown colour, deepest in the neighbourhood of blood vessels. All the lobes present a uniform appearance of a coarse reticulum, the finer bronchioles remaining distended. The bronchial mucous membrane is smooth and does not appear congested. No pneumonic consolidation or demonstrable gross oedema or hypostatic congestion is observed. No hæmorrhages or focal lesions are visible.

The pulmonary arteries and veins and their larger branches are greatly engorged with red clot which show conspicuously on the cut surface. The interlobular fibrous tissue strands, especially near the hilum stand out slightly as though swollen.

Some of the large bronchi in the lung of T.R. (Case 18) show a slight surface deposit. The appearance of the sample from M.Br. (Case 16) justifies the opinion that the description given applies also in that case.

Microscopic.—The pleura shows no lesion. The parenchyma shows a varying distribution of areas of infundibular and alveolar distension (acute emphysema) grading to areas of relative collapse. Where the distension is great the walls are thin and bloodless ; where it is absent they are somewhat swollen, the extreme engorgement of the capillaries giving the alveoli an irregular contour. Oedema is present in tissue and in alveolar spaces chiefly where congestion is most developed, but is not general. The great vessels everywhere are extremely engorged and even in the emphysematous areas wherever the tissue is sufficiently loose or the vessel large enough,

engorgement is evident. In the engorged areas occasional red blood corpuscles are seen in the alveoli, but apart from this and a few desquamated cells no cellular accumulations are seen. The bronchioles are distended and empty and their epithelium flattened, but mostly intact. The interlobular connective tissue shares in the congestion and is but slightly oedematous.

The alveolar congestion and oedema is most prominent in the lung of M.Br. (Case 16). Emphysematous distension is almost exclusively predominant in T.R. (Case 18) and in this case more congestion and oedema, and some catarrhal exudation is present in the bronchi. In W.F. and T.R. (Cases 1 and 18) a few very small congested subpleural and para-arterial areas of lymphoid tissue are present.

Lymphatic glands.—From both W.F. and T.R. (Cases 1 and 18), glands from the root of the lung, porta hepatis and lesser curvature of the stomach were examined, and from T.R. (Case 18) alone glands from the head and upper border of the pancreas and from the mesentery.

The lymph glands in all these situations show a moderate degree of enlargement. The largest gland observed was the lowest of the ileocaecal group in the mesentery and measured 2 x 1.1 x .6 cm. Commonly the glands are about half this size. On section vascular engorgement can be seen in the bronchial glands. Those from other parts are of a uniform pale pink colour with no gross evidence of congestion, hæmorrhages or focal lesions.

Microscopic.—Lymph gland—from porta hepatis. Section including the hilum of the gland shows some dilatation of the lymphatics in that situation. The afferent lymph paths, peripheral and trabecular are broadened, of loose structure and loaded with polymorphonuclear leucocytes. Many of these show nuclear degenerative changes. In these areas and in the neighbouring lymphatic tissue there are very numerous large cells with vesicular nuclei, apparently locally proliferated (some show double nuclei). They are often vacuolated and contain polymorphic nuclear debris, partly derived from lymphocytes. Some are full of blood pigment. There is much fibrin in these areas, and much loss of definition of cytoplasmic structure and loss of staining character (as contrasted with neighbouring tissue). The lymph follicular tissue is hyperplastic and slightly hyperaemic and for the most part rather rarified as though oedematous, with localized peripheral areas of denser lymphocytic accumulation. "Germinal centres" are not prominent. No polymorphs are seen in the lymphatics at the hilum.

Bronchial glands.—These show similar features but in addition are much more congested. The subcapsular lymphatic spaces are not so easily observed, lymphoid tissue frequently reaching the capsule. The internal structure of the gland is, however, much more grossly disturbed. All the vessels, arterioles, veins and capillaries are widely distended with blood. The lymph cords are represented by indefinite lymph cell areas in the medulla, practically the whole of which has the openwork appearance of the lymphatic paths. In the reticular spaces, the large endothelial cells are specially numerous. Polymorphs are also very numerous but rather more indeterminate in distribution and more patchy in concentration than above described. The concentration of polymorphs in some parts resembles that of an acute pyogenic focus. In a few areas the shadows of numerous red cells are seen in the sinuses and some even amongst the lymphocytes. Lymphoid hyperplasia is only seen at the cortical periphery, and often in the centres of the follicles nuclear fragmentation and degeneration and cytoplasmic changes recalling those seen in the splenic lymphoid tissue are present.

Ileocaecal lymph glands.—These vary somewhat in the appearances presented but all are swollen and show marked vascular engorgement throughout but no hæmorrhages. The lymph sinuses are dilated and in some rather empty with somewhat less prominent endothelial swelling, desquamation and phagocytic activity than that seen in the bronchial glands; others are quite as active as those glands in this respect. Some of them show numerous curious degeneration forms, apparently of lymphocytic nuclei, which make the cells difficult to distinguish from polymorphs. These are especially numerous round dilated blood vessels. The lymph cords are wide, the reticulum delicately spread out and the lymphocytes uniformly and somewhat loosely distributed. The peripheral lymph follicles for the most part are not well marked off, and show some endothelial activity but only occasionally are there seen the degenerative changes resembling those in the spleen. Other smaller abdominal glands are less affected and show chiefly an œdematous condition together with some vascular congestion.

Thymus.—This organ was examined in two cases—W.F. and T.R. (Cases 1 and 18).

	W.F.	T.R.
Weight	15·5 gm.	14·7 gm.
Dimensions	5·5 x 4 x 1 cm.	6·4 x 4 x ·8 cm.

The organs show no notable pecularity in form or position. Some slight congestion of superficial veins is seen. No hæmorrhages are visible on the surface or on section. The lobules on section have a pale pink colour and are free from obvious lesions.

Microscopic.—All the blood vessels are considerably engorged but no hæmorrhages are present. The whole thymic tissue is of very loose texture and wide spaces are present between groups of cells. The cellular elements are of healthy appearance and no hyperplasia is observed. No areas of hæmorrhage or necrosis are seen ; no nuclear fragmentation or phagocytosis and no abnormal cellular accumulations.

Brain and Meninges.—The whole brain was retained from T.R. (Case 18) and some small pieces from M.Br. (Case 16). Portions of the dura mater were examined in each case.

Macroscopic.—The dura mater is not thickened, the surfaces show no notable alteration and little indication of vascular congestion is seen. The superior longitudinal sinus contains red clot and anteriorly on the left side appears to communicate with an oval area of blood clot 2 x 1 cm. in extent in the layers of the dura. The specimen is parchment-like and it is difficult to estimate the true nature of the appearance.

The Brain.—Over the whole surface and in the sulci the vessels of the pia mater are much engorged, even minute branches being affected. No surface hæmorrhages of macroscopic size are present. The preservation makes any estimate of the internal condition of dubious value. No grossly recognizable changes are visible. A little pigmentary diffusion into the brain substance round the blood vessels is seen, but no hæmorrhages.

Microscopic.—Brain and Meninges.—All sections show the effect of postmortem change and shrinkage. Representative sections were examined from all important parts of the specimen from T.R.

Vascular congestion is present in the pia mater and extends along the sulci, but only a very few mononuclear and lymphocytic cells are seen outside the vessels and no hæmorrhages are present.

The brain tissue shows many rounded and irregular spaces of artificial origin especially the mid brain. Fine histological evidence of the condition of the ganglionic cells is lacking as nuclear and granular definition is poor and no observations are possible on the dendrites. Between these cells the rest of the sections show mainly a granular looking reticulum with darkly staining nuclei dotted through it. Dilatation of the blood vessels is present in many parts, more especially near the surface of the cerebral hemispheres and in the meninges where minute capillaries are engorged.

In the specimens from T.R. no evidence of escape of blood from the vessels can be seen in any part, but in those from M.Br. (Case 16) red blood corpuscles are found in small numbers outside the vessels in the cortical tissue.

There is no evidence of any cellular accumulations round the blood vessels, ganglionic cells or elsewhere in the substance of the brain. Necrosis is not seen in any part.

Heart.—The complete organs from W.F. and T.R. (Cases 1 and 18) and the apical portion of both ventricles from M.Br. (Case 16) were examined. No part of the pericardial sac was available.

	Weight.
W.F. (Case 1)	50 gm.
T.R. (Case 18)	70 gm.

Macroscopic.—Through the unaffected epicardium is visible a marked degree of venous congestion that involves the injection of many tiny venules. No petechical hæmorrhages are observed. The atria have been damaged during removal, but as far as can be judged the chambers were not dilated. The left ventricle is firmly contracted. In T.R. (Case 18) a little friable red clot is found in both atria, the upper part of the left ventricle and the aorta, and red clot fills the right ventricle and extends into the pulmonary artery. A little fibrinous material in the right ventricle and a little red clot in the left atrium and the pulmonary artery is all that was left in the specimen from W.F. (Case 1) which has been opened at autopsy. No petechiæ, adherent thrombi, or affection of the endocardium is found in any of the chambers. The valve cusps and chordæ tendineæ appear unaffected. Irregular blood staining of the endocardial surfaces is present in all chambers and the valve cusps and the interior of the aorta are similarly discoloured. The thickness of the right ventricular wall near the base is ·3 cm., that of the left ·9 cm. No hæmorrhage or focal pathological change is observed in the muscular tissue, but some vascular congestion can be seen. The appearance of the specimen from M.Br. (Case 16) indicates that the heart was in a similar condition to those described.

Microscopic.—In no part of the heart can any change of a pathological nature be found in the epicardium or endocardium. There is much congestion of the superficial arteries and veins and even of vessels in the muscle, but no hæmorrhages are found. No swelling, loss of striation, hyaline or fatty change or other indication of pathological alteration is recognizable.

In the case of M.Br. (Case 16) " fragmentation " of the muscle fibres in the left ventricle is observed. The connective tissue shows no abnormal features. In the sections from W.F. (Case 1) in a few places in the perivascular and subepicardial connective tissue there are very small collections of lymphocytes. No hæmorrhage is found in the muscular tissue.

Valves.—No change is found in the endothelium. No vascularity or cellular infiltration is seen. There is no indication of endocarditis.

Arteries, veins and capillaries.—No significant changes are seen in the walls of any of these structures which have been examined except as recorded in the spleen, where some hyaline change is seen in some of the arterioles (most marked in J.P. (Case 12)). Gross affection of the endothelium is not observed in the vessels of any organ.

Thrombus from inferior vena cava.—This was removed at autopsy from Case 12 and was not adherent to the vein. Similar thrombi are described as being constantly present in superior and inferior venae cavae in the other cases.

It is apparently moulded to the form of the vessel in a partly collapsed state. In transverse section measures 1·25 c.m. x ·6 c.m. The preserved specimen is greyish white externally and material of this colour constitutes most of its bulk. In the middle it consists of dark red clot conforming to the shape of the mass and forming a central canal of rather less than $\frac{1}{3}$ of the whole in sectional area.

On microscopic examination the outer portion consists of leucocytes and fibrin, the inner of red blood corpuscles.

A differential count of the white blood corpuscles shows—Polymorphs 27%, lymphocytes 52%, and large mononuclears 21%.

BACTERIOLOGICAL EXAMINATION OF SECTIONS OF THE AUTOPSY MATERIAL.

Organisms were diligently sought for in sections of all the organs from each case and of the clot obtained at autopsy from the inferior vena cava of J.P. and from the heart and blood vessels wherever possible.

J.P. (*Case* 12).—In the clot, chiefly along the surfaces, internal and external, of the fibrinous portion are seen abundant gram positive bacilli of putrefactive type, abundant staphylococci and less numerous streptococci. The bacilli are the most diffusely distributed. The staphylococcal groups which are most numerous on the external surface are in a number of places colonies of some size. A small colony of streptococci, the largest group of this organism found, is present in the red clot.

In the arteries of the kidney and in the capsule are numerous organisms, gram positive bacilli (some of putrefactive type), gram negative bacilli and a few streptococci and staphylococci. There is no apparent reaction to their presence and only in a few instances are they seen in the tissues immediately beyond the walls of the vessels. On the peritoneal surface of the liver similar organisms are seen. In the substance of the liver a few gram positive bacilli and a few doubtful coccal forms are seen. On the surface of the spleen are gram positive bacilli. None were encountered in the tissues of that organ and cocci were not certainly identified.

W. F. (*Case* 1).—In this case the only place in which organisms are found in any numbers is the capsule of the kidney where gram positive cocci (staphylococci) and bacilli are present. In the clot from the left atrium gram positive bacilli (putrefactive), gram negative bacilli and gram positive single cocci and diplococci are present. On the mucous surface of the stomach in the debris are numerous organisms, bacilli and cocci, which invade the tissue scarcely at all. In the other tissues only occasional gram positive bacilli and coccal forms liable to suspicion are seen.

M. Br. (*Case* 16).—In every tissue gram positive putrefactive bacteria are numerous. Coccal forms are seen but are not numerous and always leave their identification uncertain.

T. R. (*Case* 18).—The same appearances are present on the mucous surface of the gastro-intestinal tract as have been described in W. F. (Case 1). At no point is more than superficial invasion observed nor is reaction to their presence excited.

In the kidney capsule a few doubtful gram negative bacillary forms are present. In the exudate on the bronchial surfaces, organisms in great variety and abundance are seen. In the rest of the organs bacteria are difficult to find, and the same doubt of coccal forms seen makes such observations as are made of no value. No organisms are found in the blood clots in the heart.

Smears from organs and films from plate cultures from the heart blood and pericardial fluid obtained at the autopsy on M.Br. by Dr. Richards and stained at his laboratory were available for examination.

Smear from *Spleen.*—In this film are seen abundant gram positive bacilli of putrefactive type many of them spore bearers, a very few diplo-streptococci and staphylococci and a few faintly staining negative bacilli.

Smear from *Lung.*—Cells from the bronchial mucous membrane are recognizable. The film contains abundant organisms ; gram positive baccilli of putrefactive type, short slender and longer and thicker gram negative baccilli, long slenderer filamentous gram negative forms, gram negative fusiform baccilli, gram negative cocci, abundant gram positive streptococci, and scantier staphylococci and gram positive diplococci resembling pneumococci.

Films of colonies from the plate culture of the *heart blood.*—These show a small gram positive bacillus of diptheroid morphology, some longer and thicker gram positive bacilli and large pleomorphic gram positive diplococci with the individuals of pairs showing flattened adjacent surfaces.

Films of colonies from the plate cultures of *pericardial fluid.*—These show the same organisms as those from the culture of the heart blood and, in addition, a smaller rounded gram positive coccus of diplococcal habit and staphylococcal grouping, possibly only a variant of the larger form.

COMMENTARY ON BACTERIOLOGICAL AND PATHOLOGICAL FINDINGS.

Bacteriological.—The rapid development of putrefactive and other bacteria in the tissues is not surprising in view of the temperature conditions at Bundaberg, but whether the invasion began terminally or post-mortem, it is not now possible to establish by histological examination that a particular organism caused the deaths. No plugging of vessels in the kidney or elsewhere, nor focal concentration of any one pathogenic organism in the tissues with or without lesion is to be observed. Nor when the route of entry of the organisms and the rapidity of death is considered, even on a hypothesis of staphylococcal septicaemia need this cause great surprise. It is notoriously difficult to demonstrate cocci in the tissues of animals inoculated intravenously with lethal doses, if death follows too rapidly for the development of local lesions. In the absence of such lesions, for the occurrence of many cocci in the amount of tissue on a microscopic slide, the distribution of enormous numbers in the whole body would be required. It is difficult to be absolutely certain of the diagnosis of gram positive cocci singly or in pairs in the presence of the numerous artefacts in such post-mortem material scarcely to be distinguished from them, and while we may be convinced that all the forms observed cannot be dismissed as artefacts, they can in the circumstances have no weight as evidence. We have, therefore, no unequivocal histological evidence that staphylococci (or another organism) multiplied in vivo in the blood stream or in the tissues. On the other hand, the evidence can not be taken as excluding either hypothesis. There is, of course, no means of proving that any staphylococci observed are identical with the Bundaberg staphylococcus.

Pathological.—Though the detailed account of the pathological findings does not provide us with conclusive evidence of the cause of the deaths of these children, it does put us in possession of valuable information in the light of which the nature of those deaths may be discussed, and by which the validity of the various hypotheses put forward on other grounds may be tested.

The uniformity of the findings confirms what is not seriously in doubt, the unity of the cause. It also increases the probability that they are, with the possible exception of those in the gastro-intestinal tract, safely applicable to the cases which were not examined in detail. We are concerned here with an agent so rapidly lethal that there is barely time for the occurrence of appreciable lesions, and insufficient time for the development of characteristic changes which might permit specific diagnosis.

Nervous and Cardiovascular Systems.—That in common with other tissues the central nervous system was profoundly affected by the action of a poison is abundantly evident from the effects on cerebral and motor functions, and heat-regulatory and cardiovascular control.

We can point to no primary changes in the nervous system, central or peripheral, to correspond with the neurotoxic symptoms exhibited. The cerebral congestion was probably exaggerated by the convulsions in the cases in which these occurred. Though it is scarcely possible that it can have been entirely unaffected, the heart muscle is to all appearances sound. Except for the slight changes in the splenic arterioles, no demonstrable lesion is found in any of the blood vessels. Yet the poison, however produced, vitally affected the neuro-cardiovascular mechanism. Of this we have evidence in the blotchy cyanosis of the skin and in the peripheral congestion in the organs, as well as in the condition of the pulse. It is probable that the blood pressure was low.

The absence of petechial hæmorrhages in a toxaemia of this severity is somewhat surprising. They were not seen at autopsy or found later. The escape of a few red cells from the vessels is seen in the lungs and at the surface of the brain in M.Br. (Case 16). These are probably related to the pulmonary congestion and the convulsions. More such microscopic hæmorrhages might have been observed had the conditions for the preservation of the erythrocytes been more satisfactory.

Respiratory System.—Information on changes impairing the capacity of the blood to effect gaseous exchanges is necessarily lacking. There may have been primary stimulation of the respiratory centre. The increased respiratory rate and the occurrence of areas of acute emphysema in a greatly congested lung suggest response to a severe anoxaemia. There is no evidence of obstruction of the respiratory passages.

In the Liver, apart from the general stasis, which is evident on both sides of its venous circulation and in its uniform sinusoidal congestion, and from the moderate toxic effects on its cells, the variable slight leucocytic infiltration of the portal tracts is the only change of note. This infiltration, with the presence of occasional polymorphs and of cells showing nuclear degenerative changes, together with the appearance of the lymph glands from the porta hepatis is one of the few localized effects observed and is possibly significant.

In the Kidney there is again congestion, in places extreme, and a more marked toxic effect especially on the epithelium of the convoluted tubules. Infarcts, necroses, cellular infiltrations, hæmorrhages, and other lesions are absent.

The Suprarenal is to all appearances completely normal.

Intestinal Tract.—The possibility of gastro-enteritis from "food poisoning" or the ingestion of some metallic or other irritant must be considered. It does not appear to us that the affection of the bowel, which mainly falls on lymphoid tissue, is of an extent or degree that will support any such theory. The slight amount of congestion, oedema and leucocytic infiltration in the mucosa, the absence of hæmorrhages, necrosis, ulceration or bacterial invasion of mucosa or lymphoid tissue, and the nature of the changes in the liver and kidneys (particularly in the latter case the slight affection of the epithelium of the convoluted tubules), are unfavorable to the supposition that gastro-enteritis of any kind was the affection responsible for the deaths. The vomiting, intestinal disturbance and biliary flow and the condition of the tract, are amply accounted for by the action of a circulating poison on the central nervous system, the lymphoid tissue and secretions of the tract, and on the vascular system.

The Lymphoid Tissue.—Both macroscopically and microscopically the changes found in this tissue in the lymph glands, spleen and intestinal tract are the most definite and striking of any observed. Lymphatic tissue in children is frequently prominent and readily affected by injurious agents arriving by the blood or lymph paths.

That a toxic agent circulating in the blood stream was acting is obvious from the degenerative changes seen in the lymphoid tissue of the spleen and the similar appearances in the follicles of the lymph glands and in the Peyer's patches. In the lymph glands, the nuclear changes common in the lymphocytes, the dilatation of the sinuses, the endothelial hyperplasia and phagocytosis and the presence of occasional polymorph accumulations in the sinuses, afford evidence of toxic action. The presence of fibrin in the sinuses of some glands (bronchial) is evidence of the severity of the action of the poison. The appearances in the bronchial glands and those from the porta hepatis suggest its arrival by the lymph paths, though no focus for drainage in the case of the bronchial glands is observed and that in the liver is uncertain. The extreme congestion in the bronchial glands is probably in part due to the same causes as that in the lungs.

General infections (scarlatina, diphtheria) are common causes of lymphadenitis. Much attention has been paid to lesions of lymphoid tissue in diphtheria both in man and animals, and the characters of the changes observed in this disease are very similar to those which have been described in these children. There is, however, no specific diagnostic lymphatic lesion characteristic of diphtheria, but in view of the possibility of the injection of diphtheria toxin being the cause of the fatalities at Bundaberg the pathological evidence for and against this hypothesis must be examined.

All observations on autopsy material in human beings are necessarily on cases in which the disease has been present for some days, and in the case of the deaths at Baden which were held by Grassberger to be due to the injection of diphtheria toxin, death did not occur earlier than the fourth day. The reports on the autopsies of the Baden children are very incomplete, but characteristic hæmorrhagic lesions in the suprarenal are reported to have been present in every case. It is reasonable to expect that this lesion, or at least congestion, would have been present in such acute cases as those at Bundaberg, though it is conceivable that the time was

too short even for this. No hyaline change or other lesion is present in the medulla. Fatty change in the heart muscle, which is stated to occur early in diphtheria is not observed, nor is there any hyaline or other necrotic lesion, nor is interstitial myocarditis present. No hæmorrhages are seen in or about the lymph glands. The congestion in the lungs and the slight indications of toxic effects in the cells of the liver and convoluted tubules of the kidney, though they occur in diphtheria, are of no diagnostic value. Unfortunately the lesion at the site of inoculation was not observed at autopsy. In the Baden cases the infiltration about the site of injection was of such character and extent even within 24 hours that it would probably not have failed to have attracted attention at autopsy in the Bundaberg cases had it been present.

Since none of the changes, including those of the lymphoid tissue, specifically point to diphtheria toxin as the cause, while others that might be expected to be present in such acutely fatal cases are lacking, the weight of evidence may fairly be considered to be against this hypothesis.

There is no suggestion from the examination of the *Thymus* and other organs that lymphatism was a factor in the deaths of either of the cases in which this organ was examined.

Tetanus has been suggested as a possible cause. For this the examination of the tissues can give no positive assistance. Changes in the cells of the central nervous system whether of nuclei, granules or dendrites could not be satisfactorily demonstrated on the material available, and in any case none of the changes would characterize tetanus specifically. Convulsions might conceivably account for cerebral pulmonary and other congestions. Affections of lymphoid tissue form no part of the picture of tetanus poisoning. Hæmorrhages or necrosis in voluntary muscle were not specially sought at autopsy, and if present did not attract attention.

There remains the view that the staphylococcus, which was present in the bottle and is of pathogenic type, was responsible for the deaths. In spite of certain difficulties it is not only the most obvious explanation of the tragedy, but the one with which the pathological findings fit most completely.

The main assumption required is that the organisms after introduction multiplied within the body, with little lag, sufficiently rapidly to produce the necessary toxin. The question of the presence or absence of septicaemia is quite subsidiary. We have under consideration a series of cases in which the outstanding feature is an overwhelming toxaemia acting on all tissues, producing in the short time of its action demonstrable lesions in some, and in others only functional changes. Vitally important was the action on the neuro-cardiovascular mechanism, the consequences of the derangement of which we have observed, but in the elements of which no lesion was discovered. The cells of the liver and kidney show changes scarcely to be termed severe, which are common in many toxaemias. The lymphoid tissue presents the grossest damage. In the portal tracts of the liver the process is indefinite, but there are reasons for considering that it represents an early stage of reaction to toxin or possibly infection arriving by way of the blood stream. All these are early changes and, what reaction there is, is quite local, the only evidence of the advent of leucocytes from without being in the portal tracts and certain lymph glands, and then in very limited numbers. The blood as observed in the vessels contains no excess of polymorphs, but rather a preponderance of cells of mononuclear type. This may be regarded as evidence of the overwhelming nature of the toxaemia.

Can the staphylococcus produce such changes as are seen in these cases? That the staphylococcus can produce the common toxic changes in the liver, kidney and other organs is well known. It is also known that it belongs to the group of organisms which by actual invasion or by their toxins alone produce lymph glandular lesions, and further that its poison produces symptoms referable to action on the central nervous system.

The results of the examination of the tissues of rabbits killed by intravenous injection of a sufficient dose of the Bundaberg staphylococcus to cause death within 24 hours are of interest in this connexion. Ignoring the vessels plugged with cocci which are common in the kidney of rabbits so inoculated, in a favorable case (judged by the lack of reaction round such coccal plugs), the vascular and parenchymatous changes in the liver, the cellular infiltrations in the portal tracts, the swelling and the degenerative changes in the lymphoid tissue generally, the cerebral congestion, and even the congestion, slight oedema and emphysema of the lungs make a picture which has striking features of resemblance to those seen in the autopsy material from these children. In more advanced cases, from the development of focal lesions in the liver, kidneys and elsewhere, the resemblance is lost. Considering, therefore, the number of organisms and the route by which they were introduced in the inoculations at Bundaberg, and the effect this would necessarily have on the manner of their distribution, the absence in the kidneys of the children of vessels plugged with cocci is not very surprising and the resemblances are the more striking.

In postulating that the deaths were due to the introduction of living agents, the fulminating character of the infection, whatever the micro-organism, demands the satisfaction of the same requirements of virulence and pathogenicity. The effects observed in the tissues are early, and non specific within a broad group of general infections. Within that group strong circumstantial evidence implicates the staphylococcus. The hypothesis that the inoculation of virulent staphylococci was responsible for the deaths appears to be best adapted to the pathological evidence.

APPENDIX 12.

ANALYSIS OF ORGANS FOR HEAVY METALS.

For this purpose we had available only material which had been preserved for histological examination. This had been preserved first in spirit, but later it was placed in formalin and transported to Melbourne in soldered kerosene tins. On arrival here pieces of tissue were taken for histological examination, and the remainder was stored in formalin which was later found to have been contaminated with arsenic.

The results of the analysis were as follows:—

——	——	Weight.	Treated.	Amount of Metal Detected or Recovered.
W.F. Liver ..	Sample I.	100 gms.	Ashed with CaO	0·06 m.gm. arsenic recovered
	Sample II.	100 gms.	Ashed with CaO ..	0·1 m.gm. arsenic recovered
	Sample III.	100 gms.	Treated with H_2SO_4 and HNO_3 and ashed	Minute traces of lead and copper found
	Sample IV.	50 gms.	Treated with HCl and $KClO_3$ and ashed	No heavy metals found
T.R. Liver ..	.. ..	290 gms.	Treated with H_2SO_4 and HNO_3 and ashed	No heavy metals found

The formalin was removed from T.R. liver by soaking it in 50 per cent. spirit before analysis. The lead found in W.F. liver, Sample III., was probably due to a small trace of solder falling into the specimen when the kerosene tin containing it was soldered up. Pure commercial formalin contains traces of copper and of iron and the trace of copper found in this specimen probably came from this source.

The absence of these metals in the much larger sample of T.R. liver analysed was doubtless due to the formalin having been removed before the analysis. The arsenic in W.F. unquestionably came from contaminated formalin.

Arsenic is extensively used in the preservation of museum specimens and the formalin was contaminated by the hands of an attendant. All the formalin in the room was found by analysis to contain traces of arsenic. In any case the amount found was not significant.

APPENDIX 13.

THE PREPARATION OF BATCH 86—DIPHTHERIA TOXIN-ANTITOXIN MIXTURE.

This batch was prepared by Mr. R. A. Newton, the Technical Assistant in charge of the Diagnostic and Veterinary Departments at the Commonwealth Serum Laboratories. The mixture of toxin-antitoxin was made on 5th September, 1927, and filtered on 6th September, 1927. After the animal and sterility tests had been completed, it was sent to the bottling department on 10th October, 1927.

The detail of its preparation and testing is described in full by Mr. Newton, the description being an expansion with explanatory notes of the actual copy of the official notes of preparation which appears in the Minutes of Evidence.

Details of Preparation of Toxin-Antitoxin Mixture B. 86.

This was prepared from the same toxin and antitoxin used in the preparation of B. 78.

Standardization of the Toxins.

Toxins No. A. 21 (*c*) and A 21 (*b*) mixed together. Volume 4.5 litres approximately 1st June, 1927. The toxin was tested for the L+ dose against standard antitoxin from U.S.A., Hygienic Laboratory, Washington.

1st Test.

Pig No. 1, 250 gms. given .. One unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 10 = 2 c.cm.
Sterile saline = 1 c.cm.

Pig No. 2, 250 gms. given .. One unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 10 = 2.5 c.cm.
Sterile saline = 0.5 c.cm.

Pig No. 3, 250 gms. given .. One unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 10 = 2.75 c.cm.
Sterile saline = 0.25 c.cm.

All pigs alive and well at 96 hours.

2nd Test.

Pig No. 1, 250 gms. given .. One unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 2.5 c.cm.
Sterile saline = 0.5 c.cm.

Pig No. 2, 250 gms. given .. One Unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 2.5 c.cm.
Sterile saline = 0.5 c.cm.

Both pigs dead in about 40 hours.

3rd Test.

Pig No. 1, 250 gms. given .. One unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 2 c.cm.
Sterile saline = 1 c.cm.

Pig No. 2, 250 gms. given .. Same.

Both pigs dead in about 40 hours.

4th Test.

Pig No. 1, 250 gms. given .. One unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 1.5 c.cm.
Sterile saline = 1.5 c.cm.

Pig No. 2, 250 gms. given .. One unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 1.625 c.cm.
Sterile saline = 1.375 c.cm.

Pig No. 3, 250 gms. given .. One unit Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 1.75 c.cm.
Sterile saline = 1.25 c.cm.

Pig No. 4, 250 gms. given .. One unit of Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 1.875 c.cm.
Sterile saline = 1.125 c.cm.

Pig No. 1 alive and well 120 hours.

Pigs 2, 3 and 4 dead in 72 hours.

Toxin was now filtered through a Seitz E. K. special filter.

5th Test.

Pig No. 1, 250 gms. given .. One unit Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 1.5 c.cm.
Sterile saline = 1.5 c.cm.

Pig No. 2, 250 gms. given .. As No. 1.

Pig No. 3, 250 gms. given .. One unit Standard AT = 1 c.cm.
Toxin diluted 1 in 5 = 1.625 c.cm.
Sterile saline = 1.375 c.cm.

Pig No. 4, 250 gms. given .. As No. 3

Pigs Nos. 1 and 2 alive and well, 120 hours.
Pig No. 3 died, 80–90 hours.
Pig No. 4 died, 96–100 hours.
L + dose = 0.325 c.cm.

Explanation.

To find the L + dose given to any pig, divide the amount of toxin given by the dilution of the toxin.

STANDARDIZATION OF ANTITOXIN CONCENTRATED.

Commenced 27/6/27.

Batches 321 and 742/1 mixed against the mixture of toxins A 21 (*c*) and A 21 (*b*) the L + dose of which was found to be 0.325 c.cm. as previously shown.

To avoid error in measuring small volumes the antitoxin was always diluted 1 in 1000, and the toxin 1 in 10 (so that 3.25 c.cm. = one L + dose).

Test 1.

Pig No. 1, 250 gms. given .. One L + dose of toxin = 3.25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 1.0 c.cm.
Saline = 0.

Pig No. 2, 250 gms. given .. One L + dose of toxin = 3.25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0.909.c.cm.
Saline = 0.

Pig No. 3, 250 gms. given .. One L + dose of toxin = 3·25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0·833 c.cm.
Saline = 0.

Pig No. 4, 250 gms. given .. One L + dose of toxin = 3·25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0·769 c.cm.
Saline = 0.

Pig No. 5, 250 gms. given .. One L + dose of toxin = 3·25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0·714 c.cm.
Saline = 0·036 c.cm.

Pig No. 6, 250 gms. given .. One L + dose of toxin = 3·25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0·66 c.cm.
Saline = 0·09 c.cm.

Pigs Nos. 1 and 2 gained in weight.
Pigs Nos. 3, 4 and 5 slight loss in weight.
Pig No. 6 dead in 48 hours.

Test 2.

Pig No. 1, 250 gms. given .. One L + dose of toxin = 3·25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0·66 c.cm.
Saline = 0·09 c.cm.

Pig No. 2, 250 gms. given .. One + L dose of toxin = 3·25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0·69 c.cm.
Saline = 0·06 c.cm.

Pig No. 3, 250 gms. given .. One L + dose of toxin = 3·25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0·714 c.cm.
Saline, = 0·036 c.cm.

Pig No. 4, 250 gms. given .. One L + dose of toxin = 3·25 c.cm. of the 1 in 10.
A.T. 1 in 1,000 = 0·74 c.cm.
Saline = 0·01 c.cm.

Pigs 1 and 3 died, 72 hours.
Pig No. 2 died, 48 hours.
Pig No. 4 lived 96 hours.

Test 3.

Pig No. 1, 250 gms. given	..	One L + dose of toxin = 3 ·25 c.cm. of the 1 in 10. AT. 1 in 1,000 = 0 ·714 c.cm. Saline = 0.036 c.cm.
Pig No. 2, 250 gms. given	..	As No. 1.
Pig No. 3, 250 gms. given	..	One L + dose of toxin = 3 ·25 c.cm. of the 1 in 10. AT. 1 in 1,000 = 0 ·74 c.cm. Saline = 0 ·01 c.cm.
Pig No. 4, 250 gms. given	..	As No. 3.

Pigs Nos. 1 and 2 died, 96 hours.
Pigs Nos. 3 and 4 alive 96 hours.

Unitage = 0 ·74 c.cm. of the 1 in 1,000 dilution of the antitoxin.
= 1 c.cm. of a 1 in 1,350 dilution.

Therefore 1 c.cm. undiluted antitoxin contains 1,350 units. In the standardization of the toxin and antitoxin the pipettes used were never changed, but washed out and dried with ether between each step.

PREPARATION OF THE MIXTURE, OF WHICH 1 C.CM. CONTAINED 1 UNIT OF ANTITOXIN AND 8/10 OF AN L + DOSE OF TOXIN.

After preparing Batch 78, 1,845 c.cm. of toxin remained, also a quantity of antitoxin, and it was decided to prepare B. 86.

Step 1.

How much of the mixture would the 1,845 c.cm. of the toxin prepare ?
2,600 c.cm. of toxin were required to prepare 10,000 c.cm. of Batch 78.

$$\frac{10,000 \times 1,845}{2,600} = \frac{100 \times 1,845}{26} = \frac{50 \times 1,845}{13} = 7,096 \text{ c.cm.}$$

Proof.

1,845 c.cm. contain $\frac{1,845 \times 1,000}{325}$ L + doses, but each 1 c.cm. of the mixture is to contain 8/10 L + dose.

Therefore the total mixture that can be prepared $= \frac{1,845 \times 1,000}{325} \times \frac{10}{8} = 7,096$ c.cm.

Step II.

Number of Units Antitoxin required = 7,096.

Therefore the number of c.cm. of antitoxin required $= \frac{7,096}{1,350} = 5 \cdot 25$ c.cm.

Step III.

Saline required = the difference between 7,096 and (1,845 + 5 ·25) = 7,096 — 1,850 ·25 = 5,245 ·75.

Step IV.

The antitoxin was mixed with 500 c.cm. of saline and mixed with the toxin, the balance of the saline was then added, using some to wash the last traces of toxin and antitoxin in. Filtered through Seitz E.K. Special Filter.

Animal Tests.

Two pigs, 250 gms. each given	..	..	.. 1 c.c. subcutaneously.
Two pigs, 250 gms. each given	..	..	.. 2 c.c. subcutaneously.
Two pigs, 250 gms. each given	..	..	.. 4 c.c. subcutaneously.

All alive and well 30 days later.

Sterility Tests.

1 c.cm. inoculated into meat tubes (2).
1 c.cm. inoculated into 100 c.cm. flasks broth (2).
1 c.cm. inoculated into glucose agar stabs (2).

All remained sterile seven days.

APPENDIX 14.

DISPOSAL OF BATCH 86.

The batch when bottled on 10th October, 1927, consisted of 100 x 10 c.cm. rubber-capped containers and 75 x 10 c.cm. sealed glass ampoules. There were 21 rejected after filling on account of specks of glass, black specks, a trace of fluff or leakage. Two were broken and two were kept as samples, leaving 150 containers to be accounted for.

The exact number of rubber-capped bottles and sealed glass ampoules cannot be ascertained, but from the analysis of the used and returned samples there appear to have been 83 rubber-capped containers and 67 sealed glass ampoules. The demand for the former is indicated by the fact that the whole 83 had been issued, but only three of the latter.

The table below shows the disposal of the batch, the issues by the Commonwealth Serum Laboratories, the issues from the Commonwealth Health Department, Brisbane, of the 54 rubber-capped bottles sent there, and finally the numbers of partly used and unused samples returned to the Commission at Melbourne. In this last portion of the table partly used samples are indicated by an asterisk, glass ampoules by the letter (A), and rubber-capped containers by the letter " R ". These designations are not absolutely certain in quite all the cases.

The Commission had of this particular batch for investigation 26 unopened rubber-capped containers and 52 unopened sealed glass ampoules. In addition they received 30 partly used rubber-capped containers and one partly used ampoule (Derham). The partly used ampoule received from Dr. Jackson, Tasmania, could not fairly be investigated, as it had been transferred for postage to an old sterile rubber-capped bottle. The history of these rubber-capped bottles and of several others of Batch 78 (which also contained no antiseptic) is of peculiar interest in view of what happened at Bundaberg. They are dealt with in detail in Appendix 17.

STATEMENT OF ISSUES, DISPOSAL AND RECEIPTS OF BATCH 86 TOXIN-ANTITOXIN, COMMONWEALTH SERUM LABORATORIES.

Issued by Commonwealth Serum Laboratories.		—	Disposal by Brisbane.		—	Receipts by Commission.		Non-receipts.
12.10.27.	Brisbane Depot ..	24	2.11.27.	State Stores Board for Children's Hospital, Brisbane	19	Dr. Bull	22 (R)*	2 (R)
21.10.27.	Town Clerk, Melbourne	12				Brisbane Depot ..	19 (R)	..
8.11.27.	Town Clerk, Melbourne	6				Ipswich City Council ..	2 (R)	..
24.11.27.	Town Clerk, Melbourne	6	4.11.27.	Dr. C. G. Williams[1] ..	1	Murgon Council ..	5 (R)	2 (R)
26.11.27.	Shire Council, Heidelberg	4	4.11.27.	Dr. G. A. McLean ..	1	Dr. Williams ..	1(R)*	..
26.11.27.	Dr. L. B. Elwell, Stanthorpe	1	7.11.27.	Dr. P. A. Earnshaw ..	1	Dr. Green	1 (R)*	..
			7.11.27.	Ipswich City Council ..	2	Dr. Reye	1 (R)*	..
29.11.27.	Brisbane Depot ..	30	13.12.27.	Dr. H. Green[2] ..	1	Dr. McLean	1 (R)*	..
1.12.27.	Children's Welfare Department, Royal Park	1	28.12.27.	Murgon Shire Council	8	Dr. Earnshaw ..	1 (R)*	..
			6.1.28.	Dr. E. G. Thomson,[3] Bundaberg	1	Dr. E. G. Thomson ..	1 (R)*	..
19.1.28.	Dr. H. V. Jackson, Stanley, Tasmania	2	16.1.28.	Dr. A. F. Reye[2] ..	1	Commonwealth Serum Laboratory	52 (A)	12(A)
	In stock in cold room ..	64		Balance on hand ..	19	Dr. Derham (Child Welfare Department)	1 (A)*	..
						Dr. Jackson ..	1 (A)*	1 (A)
						Dr. Elwell	1 (R)*	..
						Heidelberg Shire Council	..	4 (R) (?)
						Children's Hospital, Brisbane (all used)	..	19 (R)
						Dr. Randall	1 (R)*	..
		150			54		110	40

[1] Through Taylor and Elliotts Ltd. [2] Through Queensland Druggists Ltd. [3] Through Medical and Surgical Requisites.

APPENDIX 15.

WARNING NOTICE AND COVERING MEMORANDUM ADDRESSED TO DR. J. S. C. ELKINGTON, DIRECTOR, DIVISION OF TROPICAL HYGIENE, BRISBANE, FROM DR. F. G. MORGAN, DIRECTOR OF LABORATORIES DIVISION.

NOTE.

For Users of the Diptheria Prophylactic in Rubber-Capped Bottles.

This product contains no antiseptic. Toxin-antitoxin mixture is issued from the Laboratories in a sterile condition. Should, however, the rubber diaphragm be pierced for the purpose of withdrawing a quantity of fluid for injection into a patient, the contents may become contaminated and the bacteria multiply within, making the fluid cloudy and unfit for use.

The presence of antiseptic in biological products does not guarantee sterility, but inhibits the growth of organisms. It is considered undesirable to use antiseptic in the preparation as now issued.

Persons employing diphtheria prophylactic from rubber-capped containers are advised to use the whole of the product without delay, and not to treat the bottles of this preparation as they would a bacterial vaccine; that is, to withdraw successive doses from one bottle during a period of several weeks.

For economy, it is advisable to inject a number of children at the same time, choosing the size of the phial according to the total volume required. In view of the above, the Laboratories recommend the use of hermetically sealed ampoules which are ideal for keeping products in a sterile condition when unbroken.

Where rubber-capped containers are used the possibility of bacterial contamination, as described above, should be borne in mind, and the necessary precautions taken.

To be issued with all rubber-capped bottles containing toxin-antitoxin mixture.

COMMONWEALTH OF AUSTRALIA.

DEPARTMENT OF HEALTH.

Commonwealth Serum Laboratories,
Royal Park,
25th November, 1927.

27/1531.

Memorandum.

Reference: Your memorandum No. 465 of the 15th inst.

I am enclosing a copy of instructions issued with toxin-antitoxin mixture in rubber-capped bottles. The matter contained in that statement bears directly upon your memorandum.

Since the immunization against diphtheria is usually carried out in schools, the use of ampoules of suitable size in place of rubber-capped bottles should be easily carried out. A number of children would be inoculated at the same time.

The first dose recommended is one-eighth of a c.c. and a 1 x 5 c.c. ampoule of toxin-antitoxin mixture would contain approximately 40 doses. A 1 x 1 c.c. ampoule would be sufficient to give the first inoculation to eight children.

The elimination of antiseptic from the diptheria prophylactic obviates a certain amount of stinging, due to tricresol, following injection, and at the same time avoids the possibility of undesirable chemical reaction of the tricresol with the ingredients of the mixture.

The stock of ampoules held at the Brisbane Office may be exchanged for rubber-capped bottles. They will be issued with the note as enclosed.

(Sgd.) F. G. MORGAN,
Director, Laboratories Division.

Enc. Note.

The Director,
Division of Tropical Hygiene,
Department of Health,
Eagle-street,
Brisbane, Queensland.

APPENDIX 16.

EXAMINATION OF UNOPENED BOTTLES OF BATCH 86 AND OF OTHER BATCHES WITHOUT ANTISEPTIC.

After the accident at Bundaberg, Dr. Morgan (Director Division of Laboratories, Commonwealth Department of Health) at once had six samples of Batch 86 tested by culture into broth and into alkaline meat media. The cultures examined after one, two, three, four, five, seven and thirty days showed no growth, and the control tests of the media were satisfactory. At the same time two doses of 1 and 5 c.cm. were injected from each ampoule into two guinea pigs of 250 grams weight. The animals which received 1 c.cm. showed only loss of weight for 24 hours except one in which body weight had fallen to 234 grams on the first day, stayed above 250 grams to the fourth day, had fallen to 244 grams by the seventh day and was still below 250 grams by the thirtieth day, when it had developed a slight paralysis—dragging the hind legs. The animal inoculated with 5 c.cm. from the same bottle had regained his original weight by the seventh day, weighed 328 grams on the thirtieth day and was apparently normal. The other five animals inoculated with the larger dose had recovered their original weight between the second and fifth days and none showed any symptoms, having body weights of 340, 354, 380, 352, and 364 grams on the thirtieth day.

After withdrawing from stock in the presence of witnesses twelve ampoules of Batch 86, Dr. Morgan had the remainder sealed up and stored till handed over to the Commission. Of these 52 ampoules we immediately tested six taken at random (M1, M15, M25, M28, M34 and M40) both for sterility and by injection of 5 and 1 c.cm. subcutaneously into guinea pigs of 250 grams.

These animals were not weighed daily during the first few days, but showed no infiltration. They developed no symptoms, and after twenty days the body weights of those who had received 5 c.cm. were 307, 345, 327, 337, 324 and 345 grams respectively. The corresponding animals which had received 1 c.cm. weighed 332, 314, 327, 320, 288 and 365 grams. They all appeared then to be in excellent condition and there was no sign of any paralysis.

The sterility tests made into broth and alkaline meat media were also quite satisfactory.

Later we tested a further three ampoules injecting them in doses of 5 c.cm. and 2 c.cm. into guinea pigs. The results on body weight are shown in Table.

—	Original Weight.	Second Day.	Fifth Day.	Eighth Day.	Tenth Day.	Fourteenth Day.	Twenty-third Day.	Thirty-fourth Day.
M29 5 c.cm. ..	265	258	266	287	307	335	364	380
2 c.cm. ..	247	242	257	273	317	326	349	399
M38 5 c.cm. ..	260	226	267	270	296	324	369	411
2 c.cm. ..	244	232	267	259	280	288	327	351
M51 5 c.cm. ..	249	222	259	267	300	311	324	383
2 c.cm. ..	235	215	244	264	298	308	335	371

None of these animals had any infiltration at the site of injection for the first or second days and in none of them was there any trace of paralysis evident up to the fortieth day.

A number of other ampoules and rubber-capped bottles were tested for sterility and also by injection into guinea pigs prior to inoculating them with the Bundaberg staphylococcus and with other organisms. The following were so tested :—

Ampoule M 46.—Sterile, injected into a guinea pig in a dose of 1 ·0 c.cm. No infiltration or other symptoms. Weight on the twentieth day 300 grams.

Murgon Shire Council, 3 *Rubber-capped Bottles.*—Sterile. 0 ·9 c.cm. injected into a guinea pig of 250 grams. Weight on twentieth day 303 grams. No symptoms.

Brisbane Office Supply 8 (*Rubber-capped Bottle*).—Sterile. 1 ·0 c.cm. injected into a guinea pig of 250 grams. Weight on twenty-first day 313 grams. No symptoms.

Brisbane Office Supply 10 (*Rubber-capped Bottle*).—Sterile. 1 ·0 c.cm. injected into a guinea pig of 250 grams. Weight on twenty-first day 317 grams. No symptoms.

Brisbane Office Supply 13 (*Rubber-capped Bottle*).—Sterile. 1 ·0 c.cm. injected into a guinea pig of 250 grams. Weight on twenty-first day 395 grams. No symptoms.

Brisbane Office Supply 2 (*Rubber-capped Bottle*).—Sterile. 1 ·0 c.cm. injected into a guinea pig. Weight on twenty-first day 297 grams. No symptoms.

Brisbane Office Supply 11 (*Rubber-capped Bottle*).—Sterile. 1 ·0 c.cm. injected into a guinea pig. No symptoms. Weight on twenty-second day 363 grams.

Ten ampoules and six rubber-capped bottles of batch 86 were, therefore, tested by us for sterility in broth, on blood agar and in alkaline meat media which had been shown to grow B. chauvei with ease. Various doses injected into guinea pigs demonstrated that the mixture was over-neutralized and the same fact was attested by the intradermal injection of 0·2·c.cm. from a number of other ampoules and bottles of the same batch during the course of our experiments. All the unopened bottles and ampoules were perfectly clear and remained so.

In none of the unopened ampoules or rubber-capped bottles of this or any other batch of toxin-antitoxin was there any trace of turbidity. All the samples tested by us were found to be sterile. The animal experiments show that the toxin-antitoxin was, as it purported to be, an over-neutralized mixture and our bacteriological tests lead us to the conclusion that the mixture as put on the market is properly safeguarded in regard to the possibility of contamination until the ampoules are opened or the rubber cap pierced for the removal of doses.

APPENDIX 17.

RESULTS OF INVESTIGATION OF PARTLY USED RUBBER-CAPPED BOTTLES OF BATCH 86, WITH THEIR PREVIOUS HISTORY.

The results of the investigation of these bottles are shown in the table below. Except in two cases 1 or 2 c.cm. or more of the material was injected subcutaneously into a normal guinea pig of about 250 grams weight. In many cases the amount available was too small for this purpose. Owing to our early departure for Bundaberg and the demands thrown on the Laboratory Staff at the Walter and Eliza Hall Institute, we were not able to arrange for the daily weighing of all the animals under experiment. Any decrease in weight which may have occurred during the first two or three days after inoculation was, therefore, missed in many cases.

Cultures were made on to blood agar slopes, into alkaline meat broth and sometimes into glucose brain broth as well. When only a trace of material was left in the bottle, its contents were washed out with sterile broth and the media were inoculated with the washings.

Dr. Randall of Murgon used part of one rubber-capped bottle of Batch 86 on one occasion only, his practice being to use materials of that kind only once and to discard any product that had been open for more than an hour. Using Batch 78 and Batch 86, he had inoculated 166 children, three injections being given to each at seven or eight days interval. Most of the material used was from ampoules of Batch 78 and all of it was devoid of antiseptic. No ill effects followed any of the inoculations. From his single rubber-capped phial, Dr. Randall with some difficulty withdrew about 4 c.cm. without injecting air. He sterilized the top of the bottle first with spirits and then with iodine.

The following points concerning the technique are taken from a letter to Dr. Elkington in the official file of the Health Department, put in evidence by Dr. Cumpston.

He used 6, 1·0 c.cm. syringes and 36 needles kept in spirit when not in use. Each needle was used for one injection only and was then cleaned and sterilized by drawing spirit through it several times before replacing it in spirit. The needles were lifted out of the spirit and placed on the syringe with forceps. As soon as ampoules were opened their contents were at once transferred to the syringes. Dr. Randall had not received the "warning notice." His bottle was faintly turbid but sterile on culture.

Dr. Williams of Brisbane used a rubber-capped bottle of Batch 86, making injections into two children on 12th November, 19th November, and 26th November, 1927. On 17th November, three other children received each 0·5 c.cm. On 18th and 25th January he gave injections to one child and on 29th January, a first injection to another child was given. This bottle was, therefore, used repeatedly between 12th November, 1927, and 29th January, 1928. He had had one small local, but no general reactions following his injections. The remaining contents of his bottle were clear and sterile when we received it. Dr. Williams used methylated spirit as an antiseptic both for his needles and for sterilizing the top of the bottle. It was not his practice to inject air into the bottle in order to remove material from it. He was not aware that Batch 86 contained no antiseptic and had not seen a copy of the warning notice, having bought the material from an agent and not directly from the Commonwealth Health Department, Brisbane.

Dr. Green of Brisbane also used a rubber-capped bottle of Batch 86, making sixteen inoculations in six children from it between 14th December, 1927, and 25th January, 1928. He relied on methylated spirits for sterilizing the top of the rubber cap and injected an equivalent amount of air when withdrawing material in his syringe. He washed out the syringe and needles first with "boiling" water and then with methylated spirits. Dr. Green's bottle was infected with a gram positive bacillus. The mixture produced no symptoms in a dose of 1 c.cm. injected subcutaneously into a guinea pig of 250 grams. Dr. Green had had no reactions in any of the children injected. He had asked specifically for a rubber-capped bottle. He had not received the "warning notice," having obtained the bottle through an agent and not directly from the Commonwealth Health Department, Brisbane.

Dr. Reye of Brisbane was not called to give evidence before the Commission, but the following facts have been obtained from the official file of the Commonwealth Health Department from his letter to Dr. Elkington, since confirmed by telegram to the Commission. He obtained his bottle of Batch 86 on 16th January, 1928, and gave the first injection on that day. He made seven further injections on the 20th, 23rd, and 27th of January. He used acriflavine 1 in 1,000 to sterilize the rubber cap and the children's skin, but the hypodermic syringe and needle were

boiled before use. When several injections were given on the same day, the syringe and needle were boiled between each, unless the children were in one family, when only the needle was boiled. The material in his bottle when examined by us was turbid and contained three species of contaminating organisms. It was without effect on guinea pigs in doses of 1·0 and 2·0 c.cm. He also had received his bottle indirectly through an agent.

Dr. McLean of Brisbane also used material out of rubber-capped bottles making repeated injections from the same bottle at intervals. He had inoculated two patients on the 4th, 8th, and 14th of December, 1927, and had noticed one or two local reactions like a positive Schick test. His technique was to clean the top of the rubber cap with ether. The syringe and needle were sterilized with lysol washed out first with methylated spirit and subsequently with "boiling" water. He always injected an equivalent amount of air into the bottle before withdrawing the mixture. He had received his bottle direct from the Commonwealth Health Department, Brisbane, on 7th November, 1927. He had received the "warning notice" by post some time in January and had since abandoned the use of rubber-capped bottles. When we received his bottle the remaining contents were found to be sterile and without effect on a guinea pig in a dose of 1·0 c.cm.

Dr. Earnshaw injected three patients from a bottle of Batch 86, giving each two doses at an interval of five days. The top of the rubber cap was sterilized with methylated spirit or iodine and the syringe and needle by boiling. He did not inject air into the bottle to withdraw samples. He had had no ill effects following any injections other than a slight local reaction in the older children. He had purchased his bottle on 7th November, 1927, from the Commonwealth Health Department, Brisbane, and had received a copy of the warning notice some time in January, 1928. The remaining contents of his bottle were contaminated with a staphylococcus albus, but in a dose of 1·0 c.cm., the mixture produced no ill effects, when injected subcutaneously into a guinea pig.

Dr. Elwell, of Stanthorpe, injected three children from his bottle on three occasions each, the 1st, 6th and 13th of December. He sterilized the syringe by boiling, the needles by placing them in methylated spirits for fifteen minutes, and the rubber cap by filling the depression with methylated spirits for ten minutes before withdrawing the sample. He used 0.5 per cent. phenol as a diluent for the doses. The remaining mixture in Dr. Elwell's bottle was clear and sterile. He had received the bottle about the 1st of December, 1927, directly from the Commonwealth Serum Laboratories, Melbourne. He had not received the "warning notice".

Dr. Myers used a bottle of Batch 78A to inject two patients, one received three injections on 9th December, 16th December and 21st December, 1927, and the other, one injection only on 23rd January, 1928. He had received the bottle, a partly used one, without wrappings, from the Children's Hospital, Brisbane. He was not informed that it contained no antiseptic. The contents of this bottle were perfectly clear when it reached us in Melbourne. Dr. Myers' practice was to clean the rubber cap with ether, with ether and alcohol or with dilute lysol. The syringe was sterilized in one or more of these liquids.

Dr. Patterson, Superintendent of Children's Hospital, Brisbane, had used a number of rubber-capped bottles of Batch 86. Repeated inoculations were made from the same bottle which, since many inoculations were made on one day, did not last more than three or four days. Lysol was used to sterilize the rubber cap and the needles and syringe were sterilized in lysol and subsequently washed out with distilled water.

Dr. Patterson had used active immunization to control institutional diphtheria with apparently good results. In eighteen months prior to the introduction of this measure there were 53 cases of diphtheria presumably contracted in hospital, and in the last seven months there had been one only. He had observed four cold abscesses following inoculation among 1,020 children immunized. Two injections of 0.5 c.cm. of toxin-antitoxin mixture were used at five-day intervals so as to get rapid immunization.

There were no cases of ill effects resulting from the injections of toxin-antitoxin mixture. The case of one child named Nock was fully investigated by a member of the commission and by Dr. S. F. MacDonald, of the honorary staff of the Hospital. They were satisfied that the child's condition was not related directly or indirectly to the immunizing injections he had received.

Dr. Patterson had not seen the "warning notice", nor was he aware that the mixture he had been using contained no antiseptic.

Dr. J. V. Duhig, the pathologist in charge of the hospital laboratories, thought that the technique used for injections was so good as to preclude any possibility of serious contamination, particularly as the bottles were used up so rapidly. He had requested the Commonwealth Health Department, Brisbane, to let the hospital have rubber-capped bottles in place of 1.0 c.cm. ampoules which he found wasteful and expensive. He had received a copy of the "warning notice" at this time from the Commonwealth Health Department, Brisbane.

None of the remaining contents of any of the bottles used in this hospital were available for examination by the Commission.

Under the direction of Dr. Tilling, 23 children were inoculated by specially trained nurses from the contents of rubber-capped bottle of Batch 86 between the 3rd and 16th of January, 1928. Inoculations were given on 3rd, 4th, 6th, 9th, 11th, 12th, 13th and 16th January. This bottle was completely used.

Dr. Tilling was unaware that the bottles of this batch contained no antiseptic. These cases were injected at the Children's Hospital. Miss Barstow was one of the nurses who had carried out these injections. She had noted no ill effects following any of the injections. She apparently did not know that the material she was using contained no antiseptic. The technique used was to sterilize the rubber cap, and the syringe, which was kept for this purpose only, with ether. The needles were sterilized with strong lysol.

Dr. Lowe, of Heidelberg, had given up using rubber-capped bottles since "about two months before Christmas". He had, however, used part of a rubber-capped bottle of 78A (bottled 28th November, 1927). He was apparently using toxin-antitoxin mixture for the treatment of various conditions and not for immunization. It was his practice to make repeated injections from the same bottle at varying time intervals. One of his patients, a woman of 48 years of age, became very ill with vomiting and high temperature after an injection. He opened the bottle from which this injection was made and found it contaminated. He attributed this to air contamination in withdrawing samples from the bottle. The batch to which this bottle belonged is uncertain. The bottle itself was not available for examination. He used flavine to sterilize his syringe and needles, and methylated spirits to sterilize the rubber cap. Dr. Lowe did not remember any of the notices packed round the bottles inside the cartons.

Dr. A. P. Derham, of Melbourne, had used a sealed glass ampoule of Batch 86. 4 c.cm. were used on one day at the end of December, and the cut end of the ampoule was sealed with cotton wool moistened with spirits. It was his practice to use the ampoules on the second day as well as the first and even on the third day after opening them, the opened ampoule being in the meanwhile plugged with cotton wool. This ampoule was sterile when examined by the commission.

Dr. Hilda Bull who was immunizing school children for the Melbourne City Council, had used a large number of rubber-capped bottles of Batch 86 and had been warned previously by Dr. Morgan (Director of the Division of Laboratories, Commonwealth Department of Health) that they contained no antiseptic. She used 24 bottles of Batch 86. The top of the rubber-cap was sterilized by first pouring on methylated spirit and then burning it off. The three bottles labelled Bull 1, 2 and 3 and the nineteen other partly-used bottles (labelled Melbourne Health Department) tested by us, came from this source. Only five out of 22 were contaminated and eleven contained only a trace of material so that a large number of inoculations must have been made from each. No risk attached to these contaminations as it was her practice to use the whole contents within one hour and discard material that was left. She had observed no ill effects following a large series of inoculations. Growth in some of the bottles had taken place in the long period which had elapsed since she used them.

The only rubber-capped bottle containing antiseptic which appears in the table is from Dr. J. A. Goldschmid, of Brisbane, and belonged to Batch 989/3. He had inoculated two children on the 1st, 8th and 15th of December from this bottle without untoward results. The remaining contents were sterile when examined by the commission and in a dose of 1.0 c.cm. produced no ill effects when injected subcutaneously into a guinea pig.

The use of rubber-capped bottles containing toxin-antitoxin with or without antiseptic, when large numbers of children are to be inoculated on one occasion, is a very convenient one and perfectly satisfactory when the rule followed by Dr. Bull and Dr. Randall, of discarding all material not used within an hour, is adhered to. It is evident that the practice of using the same rubber-capped bottle on a number of occasions at widely-spaced intervals is very prevalent, possibly because medical men are accustomed to administer vaccines containing antiseptic in this way.

Of the rubber-capped bottles which the commission examined, 30 per cent. were shown to be contaminated. Considering the large number of injections given from them and the possibilities of air contamination, this percentage is not excessive (Appendix 4), though some of the contaminations are doubtless referable to faulty technique.

We have not examined a series of partly-used bottles containing antiseptic, but are assured from other experiments that with antiseptic present (Appendix 4), the percentage of contamination would be negligible.

RESULT OF EXAMINATION OF PARTLY USED RUBBER-CAPPED BOTTLES OF BATCH 86.

Source of Bottle.	Whether Clear or Turbid.	Cultures.	Animal Inoculations.	Result.
Dr. Thomson, Bundaberg	Turbid ..	Staphylococcus aureus	Numerous, described separately	
Dr. Randall, Murgon ..	Slightly Turbid	Sterile ..	Not inoculated	
Dr. Williams, Brisbane ..	Clear ..	Sterile ..	Guinea pig, 1·5 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 295 grams
Dr. Green, Brisbane ..	Turbid ..	Gram positive bacillus	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 340 grams
Dr. Reye, Brisbane ..	Turbid	Staphylococcus Gram positive bacillus Gram negative bacillus	Guinea pigs, 2·0 c.cm. and 1·0 c.cm., subcutaneously	No symptoms. Weights on twenty-first day, 339 grams and 338 grams
Dr. McLean, Brisbane ..	Clear	Sterile ..	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 332 grams
Dr. Earnshaw, Brisbane..	Turbid ..	Staphylococcus albus	Not inoculated	
Dr. A. P. Derham, Melbourne (Ampoule)	Clear ..	Sterile ..	Guinea pig, 4·0 c.cm., subcutaneously	No symptoms. Weight on thirtieth day, 435 grams
Dr. Elwell, Stanthorpe ..	Clear ..	Sterile ..	Not inoculated	
Dr. Hilda Bull, I., Melbourne	Clear ..	Sterile ..	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 340 grams
			Rabbit, 1·0 c.cm., subcultaneously	No symptoms by thirty-second day.
Dr. Hilda Bull, II., Melbourne	Clear ..	Sterile ..	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms except loss of weight by twenty-first day which had fallen from 234 grams to 214 grams. Weight on thirty-second day 271 grams. No paralysis
Dr. Hilda Bull, III., Melbourne	Faintly Turbid	Staphylococcus albus	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 344 grams
Dr. J. A. Goldsmid, 989/3 (containing antiseptic)	Clear ..	Sterile ..	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 319 grams
Melbourne Health Department I.	Turbid ..	Gram positive sporing bacilli. Gram negative bacilli	Guinea pig, 1·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 340 grams
Melbourne Health Department, II.	Turbid ..	Gram positive tetra coccus	Guinea pig, 2·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 395 grams
Melbourne Health Department, III.	Clear ..	Sterile ..	Guinea pig, 1·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 360 grams
Melbourne Health Department, IV.	Clear ..	Sterile ..	Guinea pig, 2·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 374 grams
Melbourne Health Department, V.	Clear ..	Sterile ..	Guinea pig, 3·0 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 400 grams
Melbourne Health Department, VI.	Clear ..	Sterile ..	Guinea pig, 0·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 358 grams
Melbourne Health Department, VII.	Clear ..	Sterile ..	Guinea pig, 2·0 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 387 grams
Melbourne Health Department, VIII.	.. ..	Gram positive and gram negative filamentous bacilli	Not inoculated ..	

RESULT OF EXAMINATION OF PARTLY USED RUBBER-CAPPED BOTTLES OF BATCH 86—*continued.*

Source of Bottle.	Whether Clear or Turbid.	Cultures.	Animal Inoculations.	Result.
Melbourne Health Department, IX.	Clear ..	Sterile ..	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 365 grams
Melbourne Health Department, X.	Turbid ..	Gram positive bacilli	Not inoculated	
		Gram negative bacilli	Not inoculated	
Melbourne Health Department, XIII.	Turbid (only trace)	Sterile ..	Not inoculated	
Melbourne Health Department, XIV.	Turbid (only trace)	Sterile ..	Not inoculated	
Melbourne Health Department, XV.	Clear (only trace)	Sterile ..	Not inoculated	
Melbourne Health Department, XVI.	Clear (only trace)	Sterile ..	Not inoculated	
Melbourne Health Department, XVII.	Clear (only trace)	Sterile ..	Not inoculated	
Melbourne Health Department, XVIII.	Clear (only trace)	Sterile ..	Not inoculated	
Melbourne Health Department, XIX.	Clear (only trace)	Sterile ..	Not inoculated	
Melbourne Health Department, XX. ..	Clear (only trace)	Sterile ..	Not inoculated	
Melbourne Health Department, XXI.	Clear (only trace)	Sterile ..	Not inoculated	

APPENDIX 18.

EXAMINATION OF THE REMAINDER OF THE TOXIN-ANTITOXIN MIXTURE IN THE BOTTLE OF BATCH 86 FROM WHICH DR. THOMSON MADE HIS INJECTIONS ON THE 17TH, 20TH, 21ST, 24TH AND 27TH JANUARY.

Subsequent History of this Bottle.—On the afternoon of 28th January, Dr. Thomson handed the bottle to the Mayor in the presence of Mr. McLeod. It was taken by him to Dr. McKeon's surgery where in the presence of Messrs. Marshall, Finnimore, McLeod, McCarthy (the Town Clerk), and the Mayor, Dr. McKeon withdrew about 0·3 c.cm. using a sterile syringe, but without sterilizing the top of the rubber cap.

This portion of the contents was placed in a small sterile rubber-stoppered phial. The original bottle and the phial were now made up in separate packages and sealed by Mr. Marshall in the presence of the above witnesses. The small phial was placed in the safe at the Council Chambers and handed over to Dr. Tilling on 31st January. Dr. Tilling took it to Brisbane and placed it in safe deposit, together with another sealed package containing the needles and syringe used by Dr. Thomson. They were forwarded by registered post to the Commission at Melbourne on the 3rd February.

The sealed package containing the original bottle was placed in the safe at the Council Chambers and handed to Dr. Murray on 30th January. Dr. Murray took it to Brisbane and handed it to Dr. Elkington who enclosed it in a second sealed package and transmitted it by post to the Health Department, Melbourne. On arrival on the 3rd February, 1928, the registered package was at once handed over to the Commission and was opened at the Hall Institute by the members of the Commission who found that the wrappings placed on the package at Bundaberg and Brisbane had not been tampered with, and that the seals were intact. The second sample arrived by post at the Hall Institute on 6th February, 1928, and when opened the seals of the original packages made up in Bundaberg were also found to be intact.

The bottle was examined on the evening of its arrival in Melbourne. It contained 1·45 c.cm. of turbid fluid, 1·25 c.cm. of which was removed, after the rubber cap had been sterilized by methylated spirit and flaming, with a syringe sterilized by boiling in water for 25 minutes. The operation of withdrawing the contents was carried out in a dust-free cabinet. 0.2 c.cm. was subsequently estimated as remaining in the bottle.

The total amount actually used in immunizing injections in Bundaberg was said to be 7.0 c.cm. With 1.47 c.cm. in this bottle and 0.3 c.cm. previously withdrawn by Dr. McKeon forming the second sample (B.T.AT. (2)) examined, there remains 1.23 c.cm. to be accounted for, or possibly slightly more if, as is usual, generous measure is given in filling the bottle. It is not unreasonable to suppose that this is fully accounted for by wastage in the dead space of the syringe and needle and in adjusting dosage when 45 separate injections on five occasions have been given. Dr. Thomson states definitely in his evidence that more was removed on each occasion than was necessary.

MICROSCOPICAL AND BACTERIOLOGICAL EXAMINATION OF THE FATAL BOTTLE.

A.—Microscopical.—Films were made immediately from the contents of the rubber-capped bottle of toxin-antitoxin used at Bundaberg (labelled B.T.AT.). When spread on glass slides a distinct acid odor could be recognized with the nose close to the slides. The films were stained by Gram's and Ziehl Neelsen's methods. They showed numerous gram-positive cocci which varied somewhat in size and arrangement. They were mostly in pairs or as single cocci, but also in short chains of four to six cocci. There was also much amorphous and granular gram-negative material. Seven films altogether were examined and in five of them a few clumps of yeast were found, but apart from the staphylococci, no other organisms of any kind.

B.—Bacteriological.—Cultures were made immediately from the aseptically removed contents of the rubber-capped bottle on or in the following media, with the results as shown :—

(1) Two (2) rubber-capped bottles of toxin-antitoxin of Batch 78A prepared recently at the Commonwealth Serum Laboratories and containing no antiseptic. 0·01 c.cm. was injected through the rubber cap into each bottle. One bottle was incubated at 37° C. and the other left at room temperature (20-32° C.), whilst a third uninoculated bottle was placed in the incubator at 37° C. The

latter remained perfectly transparent during the subsequent five (5) days, and at later dates it was still quite clear. The inoculated bottles became slightly turbid on the following day, particularly that at 37° C., and the turbidity of both was greater on the second day. On the third day some of the toxin-antitoxin was removed, films were made and also subcultures into alkaline meat medium and glucose brain broth. The films showed only gram-positive cocci, diplococci, short chains of three and four cocci, and many small groups or clusters of cocci. Films made later from the subcultures in the above-mentioned media showed a very similar picture and hanging drop preparations showed mainly diplococci, but also short chains of four cocci and clusters.

(2) Four (4) tubes of glucose brain broth. These were inoculated with one platinum loopful, one of each incubated aerobically and anaerobically at 37° C. and at room temperature. The latest pattern of McIntosh and Fildes anaerobic jars was employed for all anaerobic cultivation. Growth was rapid aerobically at 37° and acidity without gas was noted within 24 hours, whilst the same results were obtained at room temperature only on the second day. On the fifth day the medium became alkaline and on the sixth a small amount of gas was noted. Anaerobically there was more growth at 37° than at room temperature. Films from these cultures showed gram-positive cocci, diplococci, chains of three and four and even as many as six cocci, also tetrads and small clusters. These aerobic and anaerobic cultures were plated on blood agar aerobically and anaerobically and the anaerobic brain broths re-inoculated into brain broth. The blood agar plates showed numerous glistening white colonies resembling at first staphylococcus albus. A slight degree of haemolysis was slowly produced, and the colour of the colonies became slightly yellowish. There were no very small colonies where the colonies were spaced out.

(3) Four (4) tubes of glucose broth prepared at this Institute were similarly inoculated from the B.T.AT. and incubated as in 2. Turbidity and later greyish-white deposit were noted in all tubes and plating on blood agar gave only colonies of staphylococci. One small colony was picked off on to an agar slope, but gave a typical growth of staphylococcus. Films from the glucose broths showed again short chain formation as well as diplococci and clusters.

(4) Four (4) sloped agar tubes (hormone agar) incubated as in 2. Aerobically at 37° C. there was a good growth in 24 hours. It resembled staphylococcus albus, and showed the typical microscopical appearances of the staphylococci. After three days the growth was faintly yellowish, but on keeping never became typically aureus. At room temperature growth was decidedly slower. Anaerobically there was a good whitish growth at 37° C. in three days, no visible growth in three days at room temperature, but a whitish growth appeared later. Examination of films showed only staphylococci.

(5) Agar plates. One platinum loopful of B.T.AT. inoculated into melted agar, well mixed and plated (shake cultures) gave innumerable colonies. One loopful of one in ten dilution of B.T.AT. gave a moderate number of colonies, of one in a hundred dilution a few colonies only, of one in a thousand dilution very few colonies. At the time no counting of colonies was carried out, but experience obtained since by repeating this experiment with other bottles allows us to be reasonably certain that the number of living cocci in the bottle when received by us on 3rd February was not greater than 200 millions per c.cm. The whitish surface colonies grew to a considerable size and became of a slight yellowish colour in three days. The deep colonies were lenticular. Five of the smaller surface colonies were picked off on to sloped agar, but proved to be of the same type. Anaerobic plates were also made in the same way with similar results. All films showed only staphylococci.

(6) Glucose agar plates. The glucose agar was prepared at the Commonwealth Serum Laboratories. Shake cultures were made as in (5) and incubated aerobically and anaerobically. The results were much the same as in (5).

(7) Blood agar plates. Surface stroke cultures were made, using two plates each for undiluted B.T.AT. and for dilutions of one in ten, one in a hundred and one in a thousand. One set was incubated aerobically and the other anaerobically. On the aerobic plates falling numbers of colonies resembling

staphylococcus albus were found next day. After three days there was slight haemolysis. Nine colonies were picked off and subcultured. Staphylococci were alone found in films of these subcultures. There was a good growth also on the anaerobic plates and seven of the smaller colonies were subcultured on to sloped blood agar and incubated anaerobically. In 24 hours these showed whitish confluent growth or separate colonies, but films of these showed staphylococci, mostly clusters, but also diplococci and chains of three or four cocci.

(8) Sloped serum agar prepared at the Commonwealth Serum Laboratories. Stroke cultures were made with one loopful of one in a hundred and one in a thousand dilutions of B.T.AT. were made and incubated aerobically and anaerobically. The lesser dilution gave whitish discrete colonies and some confluence, the greater dilutions only discrete colonies. Two colonies of smaller type were subcultured but proved to be staphylococci. After three days the confluent growth was slightly yellowish. The anaerobic cultures gave good growths of staphylococci within three days.

(9) Glucose broth prepared at the Commonwealth Serum Laboratories. The results were much the same as in (3) and do not warrant separate description.

(10) Plain broth. Aerobic cultivation at 37° C. gave a moderate turbidity with some deposit in twenty hours, and on the third day there was a scum on the surface against the glass of the test tube. Plating on blood agar gave only staphylococci which produced haemolysis slowly. Anaerobic cultures gave only staphylococci.

(11) Alkaline meat medium prepared at the Commonwealth Serum Laboratories. One set was covered with a layer of paraffin and the other was not covered. Aerobic cultivation at 37° C. gave turbidity in 24 hours. Plating on blood agar gave only staphylococci. No gas was observed in subsequent observations up to twenty days. Films of the cultures at room temperature showed only staphylococci. Anaerobic cultivation gave only staphylococci and there was no gas production.

Some of the one in ten dilution of B.T.AT. was heated at 60° C. for 30 minutes and then incubated on to serum agar and into glucose brain broth. No growth took place within ten days.

To exclude the possibility that our media were unsuitable for the growth of anaerobic and sporing forms we inoculated control tubes with B. chauvei. Good growth was obtained with all the media tested.

Examination of sample withdrawn by Dr. McKeon—The remainder of the contents of the fatal bottle were received in a small ampoule sealed with a small rubber cork on 7th February. It was labelled by us B.T.AT. 2. As described elsewhere, the volume of the contents was very small. Indicators showed the P_h to be between 7·5 and 8. Films indicated a smaller number of organisms than in the original bottle. They were gram-positive cocci, most numerous as single cocci, but also many diplococci and less often three cocci in a row or forming a triangle. No definite chains or bacilli were seen. There was much gram-negative material, mostly in coccoid forms ; a few doubtful forms which were probably yeasts were present.

The contents were plated on blood agar. Most of the colonies were white resembling those grown from the original bottle, and there were a few smaller colonies. Subcultures of these large and small colonies were made on sloped blood agar. One large and one small colony gave a confluent whitish growth of staphylococci resembling that found in the original bottle, but one small colony gave an orange-yellow confluent growth of staphylococci. It was not known whether this latter was another contamination made in Bundaberg in withdrawing the sample from the bottle to the ampoule, a variant from the less orange type, or an air contamination of the blood agar plates.

Cultures were also made in two alkaline meat tubes and in two glucose brain broths, incubated aerobically. After two days the former were turbid and one showed a bubble or two of gas. Films, however, showed only gram-positive cocci in short chains and in groups. No bacilli could be found.

The glucose brain broths were turbid and showed acidity in two days. Films showed cocci, mostly in clusters. No bacilli could be found.

No animal inoculations were made direct from this second sample.

ANIMAL INOCULATIONS.

There was obviously an insufficient amount of material available in the original bottle for its complete characterization as a mixture of diphtheria toxin and antitoxin by subcutaneous inoculation into guinea pigs and, for reasons detailed elsewhere in this report, it seemed unlikely that excess of diphtheria toxin would prove to be responsible for the tragedy.

At a later stage on our return from Bundaberg, we used the intradermic test with and without added antitoxin to determine the presence of free toxin in some of this original material.

This was done by injecting into the skin of a white guinea pig in a total volume of 0·2 c.cm. the following doses of the material—0·02 c.cm., 0·01 c.cm., 0·005 c.cm. and 0·002 c.cm. with and without 0·002 c.cm. of standard antitoxin. None of these doses produced the slightest skin reaction, and as from 1/250 to 1/500 of the M.L.D. of diphtheria toxin gives a definite skin reaction, it may fairly be concluded that at the time when these tests were performed, nearly a month after the tragedy, the material did not contain a significant amount of free diphtheria toxin. Our subsequent experimental evidence has shown the remarkable stability of old diphtheria toxin in the presence of the staphylococcus which was isolated from the material (Appendix 25) and it may, therefore, be regarded as unlikely that significant excess of diphtheria toxin was present at any time in the bottle.

In the immediate investigation of the material for the presence of some unknown toxin of extreme potency or of a virulent living organism, we considered it advisable to test its action in as many species as possible, and to utilize intravenous injections which would afford small doses of any toxic substance present the best chance of producing fatal effects.

The animals which we used were rabbits, guinea pigs and mice. In addition to intravenous injections in these animals we decided to give to three monkeys doses comparable with those administered to the children at Bundaberg. The monkeys were said to be about three years old and weighed 4, 3·5 and 2·6 kilos respectively. 0·2 c.cm., 0·1 c.cm., and 0·2 c.cm. of the contents of the fatal bottle were given subcutaneously. The first of these animals was protected against any possible free diphtheria toxin by the subcutaneous injection three hours earlier of 6,000 units of antitoxin. None of them had any illness following the injections. Though many living organisms were shown to be present in the injected material, no abscess resulted at the site of injection. The animals were in excellent condition two (2) months later and were used again for the experiments detailed in Appendix 26.

Rabbits.—Two rabbits of 2·4 and 1·8 kilos were inoculated intravenously each with 0·1 c.cm. of the material. Both of these became definitely ill within 24 hours. One died on the fifth day with a broncho-pneumonia and septic infarcts in both kidneys from which a pure culture of a staphylococcus was obtained (identical with that isolated from the original material). The second was killed on the thirtieth day when moribund. It developed a stiff and painful left elbow joint 48 hours after injection and appeared to be definitely ill for some days. On the twenty-sixth day complete paralysis of both hind limbs developed, as has been described in experimental staphylococcal infections in this animal. At autopsy there were no obvious lesions in any of the joints, and the brain and spinal cord were macroscopically normal. The only definite macroscopic lesions were extensive organized infarctions of both kidneys, which were sterile on culture.

Guinea Pigs.—Three guinea pigs of about 250 grams weight were injected, one with ·05 c.cm. of the material subcutaneously, and two with ·03 and ·035 c.cm. intravenously. A fourth guinea pig received ·02 c.cm. of the material which had been heated to 60° C. for half an hour. The subcutaneous injection caused no trace of infiltration. The other animals which received intravenous injections were not very well for a few days and showed some loss of weight. They all survived and three of them when killed and examined nearly a month later showed no obvious lesions at autopsy. The fourth animal received an intravenous injection of 1·0 c.cm. horse serum on the fifty-fourth day when it weighed 400 grams, and no anaphylactic symptoms resulted.

Mice.—Eight mice weighing from 16 to 26 grams were injected intravenously by the tail vein with 0·1 c.cm. of a 1 in 10 dilution of the material. They showed no symptoms of illness following the injection except one weighing 16 grams in which part of the injection was given subcutaneously. This animal was very sick within 24 hours, and was found dead on the second day. No definite lesions were present at autopsy. A second mouse was found dead on the fifteenth day, and the uterus was distended with caseous material.

Two mice of 17 and 15 grams were injected intravenously by the tail vein with ·01 c.cm. of the material diluted 1–10 with saline and heated to 60° C. for half an hour. These also showed no ill-effects following the injection.

The results of these inoculations into animals are very unimpressive. There is nothing to suggest the presence of free diphtheria toxin in the bottle and its contents show, in the dosage used, no great toxicity or pathogenicity for laboratory animals. It seems probable that we are concerned either with great loss of activity of the contents of the bottle during the week which elapsed between its use on 27th January, and its examination by us on 3rd February, or with an activity which could only be manifested by injection into young susceptible human subjects. We are inclined to think that the material had lost some of its activity, that the doses we used would with advantage have been larger and administered to fewer animals, but that it is not unlikely that all the laboratory animals used were unsuitable for the purpose of testing its activity.

Subculture from B.T.AT. 2 into a bottle of toxin-antitoxin mixture (M.S.C. 3.) incubated at room temperature grew rapidly. Guinea pigs inoculated on several successive days showed no symptoms and smears of the material contained only cocci. When, however, the residual contents of the bottle were spun down and the deposit examined, two small clumps of yeast were found. No yeasts were found on any of the cultures of B.T.AT., on solid media and later attempts to grow them on Sabouraud's media were unsuccessful.

The only organisms which could be recovered from the original bottle and the sample removed by Dr. McKeon were staphylococci. The yeasts found in the original smears were not recovered by culture and were very probably an " air contamination." It is extremely unlikely that they had any pathogenic activity.

APPENDIX 19.

EXAMINATION OF THE BUNDABERG BOTTLE OF TOXIN-ANTITOXIN FOR IODINE.

We were interested in this question from the general standpoint of the possibility of the entry into the rubber-capped bottles of liquids used for sterilizing the top of the rubber cap. Iodine as used by Dr. Thomson lends itself particularly well to the investigation of this point. Other liquids used for the same purpose might quite conceivably have some deleterious action on biological products, e.g., pyridine present in methylated spirit. Apart from these considerations it seemed possible that iodine might have played some part in the accident we were investigating.

When we had withdrawn as much as possible of the remaining contents of the bottle, we asked Mr. F. H. Holden, the Research Biochemist of the Walter and Eliza Hall Institute, to estimate the amount of iodine present in the remaining contents. These contents were estimated to be 0·075 c.cm. by washing out and weighing after removing the rubber cap. The method used for iodine estimation was capable in practised hands of detecting with certainty 0·0005 m.gm. The reagents were free from iodine in quantities capable of detection by the method used. 0·003 m.gm. of iodine were found corresponding to 0·04 m.gm. per c.cm. of toxin-antitoxin mixture.

This of course represents the maximal amount of iodine ever present as it was doubtless added to by successive needle punctures.

We were not able to show that iodine in half this concentration made any appreciable difference to the growth of organisms or produced any product toxic to laboratory animals. Neither did it aid in the dissociation of toxin from antitoxin. Toxin was quite stable in its presence in this concentration. In higher concentrations it inhibited the growth of organisms without producing any toxic products in toxin-antitoxin mixture.

The presence of iodine in the bottle is also suggestive of the ease with which organisms could be carried in through the puncture in the rubber cap if the surface of the latter were not efficiently sterilized.

APPENDIX 20.

ON THE POSSIBILITY OF TETANUS BEING THE CAUSE OF THE FATALITIES.

We were not impressed in this connexion by evidence obtainable as to the symptomatology presented by the children at Bundaberg, nor did any local practitioner who saw the affected children make a diagnosis of tetanus. The microscopical and cultural examination of the bottle of toxin-antitoxin mixture used by Dr. Thomson lent no support to this view, nor were we aware of such great individual variations in susceptibility to tetanus toxin as was shown by these children to the inoculated material. As shown elsewhere, several children died within 24 hours and several showed no symptoms following an injection of 0.125 c.cm., and moreover none of the children inoculated three and six days previously suffered any illness.

The two questions we endeavoured to answer were—(1) Would the addition of a small number of tetanus bacilli to toxin-antitoxin result in the production of tetanus toxin in doses possibly lethal to children? (2) Would tetanus bacilli or their spores, having grown in the toxin-antitoxin, be demonstrable after a lapse of time, or might the bacilli multiply, produce toxin, and then disappear?

An agar culture of B. tetani (Clostridium tetani) was obtained from the Commonwealth Serum Laboratories. Films showed mostly typical drum stick forms, but also a few free spores, non-spored forms and long forms. A weak emulsion was made in normal saline and 0.1 c.cm. injected into one rubber-capped bottle (referred to later as No. 1), of toxin-antitoxin containing no phenol (Batch 86), into another (No. 2) containing 0.5 per cent. phenol (batch 111), also into two bottles (Nos. 3, batch 86 and 4, batch 111) which received in addition 0.2 c.cm. of a 1 in 10,000 dilution of an emulsion of the Bundaberg staphylococcus, an inoculation of 1,000 to 2,000 cocci per c.cm. of toxin-antitoxin in the bottle. These four bottles were placed in an incubator which was at first set at 75° F. (24° C.) and later at 80° F. (26.5° C.).

Guinea pigs were inoculated subcutaneously with 0.2 c.cm. from each of these bottles after two days, with 0.5 c.cm. of Bottles 3 and 4 after five days, with 0.5 c.cm. of each bottle after eight days, with 0.5 c.cm. of Bottle 1 after ten days, with 0.5 c.cm. of each bottle after thirteen days and after twenty days. None of these animals showed any local lesions or any signs of tetanus during the 34 days following the commencement of these inoculations. Altogether, therefore, nineteen animals were inoculated at various intervals and all survived. Their average weight at the time of inoculation was 231 grams. A few showed a slight loss of or failure to gain weight for a few days, but during the period of observation stated above the average gain in weight was 73 grams. There were no deaths among these animals, which remained in splendid condition, during a further period of 3 weeks, when they were discarded.

When the bottles were inoculated, subcultures were also made from the same agar culture of B. tetani on to agar and into alkaline meat medium. These were incubated at 37° C., the former in an anaerobic jar, and good growths were obtained which appeared to be pure when examined microscopically. Subcultures on agar incubated aerobically showed no growth. 0.2 c.cm. of a three days' culture in alkaline meat medium killed a guinea pig in two days. No infiltration could be felt at the site of inoculation. Also a guinea pig inoculated with 0.2 c.cm. of a four days' culture in plain broth was found in convulsions nineteen hours later and died after a further two and a half hours.

Subcultures were made from the bottles of toxin-antitoxin at intervals. No. 3 bottle became slightly turbid in two days, Nos. 1, 2 and 4 were still perfectly clear after six days. The turbidity in No. 3 bottle was due mainly to the staphylococcus as was shown by plating on agar anaerobically. Plating after two days gave no tetanus colonies, after five days a few filamentous forms were found amongst the staphylococci in films made from the plate, and after ten days subcultures from Bottle No. 3 into alkaline meat medium gave gas formation and both B. tetani and staphylococci were found microscopically, whereas a meat tube heated to 80° C. for fifteen minutes after inoculation gave only B. tetani. Plating from this bottle after twelve days gave a mixture of staphylococci and B. tetani. Films made from a drop of the contents showed a few spored and unspored bacilli and a few gram positive diplococci and cocci. Some of this bottle was also centrifuged and the deposit showed also B. tetani and staphylococci. In fact there appeared to have been some growth of B. tetani in No. 3 bottle, though 0.5 c.cm. did not cause any ill-effects in the guinea pig.

Subcultures into meat media from Bottles 1, 2 and 4 also gave prompt growth of B. tetani, though films made from the toxin-antitoxin and from the deposit obtained by centrifuging showed few bacilli. Finally, Bottle No. 3 (containing no phenol and inoculated with both B. tetani and the Bundaberg staphylococcus) was subcultured on the twenty-first day into a tube of meat medium and this was immediately heated to 80° C. for fifteen minutes. After several days incubation at 37° C., 0.1 c.cm. of this subculture killed a guinea pig in 43 hours, death being preceded by convulsions, whilst 0.2 c.cm. killed in 21½ hours.

We are of the opinion that the contamination by the Bundaberg staphylococcus took place at Bundaberg during inoculations on some day previous to the 27th January. It seems reasonable to suppose that a contamination by B. tetani would have occurred either at Bundaberg or at the Commonwealth Serum Laboratories. These experiments do not lend any support to a contamination by B. tetani either at the Commonwealth Serum Laboratories or at Bundaberg. With regard to the Commonwealth Serum Laboratories there is no evidence before us that any other bottle of Batch 86 or of 78A gave rise to tetanus, though large numbers of inoculations have been carried out with them. This contingency would mean that first of all the toxin-antitoxin was contaminated by B. tetani at the laboratories in November, that the bacillus began to multiply subsequently,* that the bottle was then contaminated at Bundaberg by the staphylococcus, that a lethal dose of tetanus toxin was produced only between the 24th and 27th January, so that on the 20th and 24th, for example, numbers of children could be inoculated without the slightest suggestion of tetanus toxin poisoning, and finally, that B. tetani disappeared so completely by the 3rd February that they could neither be found in films nor cultivated in suitable media.

Now in the one bottle of Batch 86 (Bottle No. 1) which received a small inoculation of B. tetani only, there appeared to be little if any multiplication or toxin production in twenty days, though B. tetani could be cultivated from the bottle by inoculating small quantities into meat medium or by plating on agar. The growth of the staphylococcus appeared to favour a slight and slow growth of B. tetani, but this was scarcely appreciable within 8 days, and there was no evidence of the production of tetanus toxin in doses lethal to guinea pigs. It was not thought necessary to look for the presence of doses smaller than this.

* With the exception of the time taken by transport to Brisbane this bottle was kept at low temperatures until 6th January. From this date to the 27th January it was kept at room temperatures averaging not higher than 80° Fahrenheit (26.5° C.)

APPENDIX 21.

IS IT POSSIBLE THAT ORGANISMS OTHER THAN STAPHYLOCOCCI WERE PRESENT IN THE BUNDABERG BOTTLE OF TOXIN–ANTITOXIN MIXTURE, WHICH HAD DIED OUT OR BEEN DESTROYED BY PROTEOLYTIC ACTIVITY OF THE STAPHYLOCOCCI?

The examination of smears of the material in the bottle of Batch 86 received from Dr. Thomson revealed the presence, in addition to enormous numbers of cocci, of a few clumps of yeasts which could not be recovered by culture. Their presence and the results of our experiments on air contamination, raised the question as to whether it was possible that some other organism had once been numerous and had first been overgrown and subsequently dissolved by the activity of the staphylococci which alone were cultivated from the bottle in a large number of media.

To test this hypothesis we inoculated a streptococcus (faecalis) and the Bundaberg staphylococcus together into a rubber-capped bottle of toxin-antitoxin mixture of Batch 86 and incubated it at 80° F. 1·0 c.cm. of the mixture was injected on the first, second, third, sixth, and ninth days into guinea pigs and in no case were any symptoms produced other than slight temporary loss of weight and local ulceration. On the tenth day both organisms were recovered by plating.

B. proteus X 19 was inoculated into a rubber-capped bottle of Batch 78A and after 24 hours the Bundaberg staphylococcus was also inoculated in a dose of about 1,000 organisms. Injections into guinea pigs in doses of 0·5 c.cm. were made on first, second, third, fourth, fifth and eighth days. Beyond some local ulceration and loss of weight no symptoms were noted, though the animal inoculated on the fifth day was very slow in recovering its original weight. After nine days incubation at room temperature (about 23° C.) both organisms were recovered by plating. Another bottle was inoculated with B. proteus X 19 alone in a dose of about 1,000 organisms. A similar series of animal injections were made without positive results. These experiments are of particular interest in that an organism with strong proteolytic powers apparently failed to cause any obvious dissociation of diphtheria toxin from toxin–antitoxin mixture.

Other streptococci tested for powers of growth and survival for ten days in toxin–antitoxin mixture were a haemolytic strain from a puerperal case and one from a case of scarlet fever. Guinea pigs were inoculated at the intervals described above and none showed any symptoms. The organisms were recovered from the bottles at the end of the experiment. Similar experiments were later carried out with B. tetani (Appendix 20). In view of these findings it did not seem worth while pursuing this line of inquiry further.

No experiments were made with yeasts as we had been unable to recover them by culture either on the many media in which the staphylococcus grew, in bottles of toxin–antitoxin, or later, on special media.

It is very unlikely that any pathogenic organisms of microscopic size other than the staphylococci were at any time present in any considerable numbers in Dr. Thomson's bottle. If, as is possible, a few other contaminating organisms got in, they should, if they had grown, have been recoverable by culture when the bottle reached us.

We cannot exclude the presence of a pathogenic filter-passing organism in the Bundaberg bottle, but it is exceedingly improbable that any such organism would have grown in so feebly nutrient a medium. The only organism other than the staphylococcus of whose presence in Dr. Thomson's bottle we have evidence, is the yeast which we were unable to recover and which did not appear to have multiplied to any extent in the bottle. Unlike the case of the staphylococcus, we cannot exclude the possibility that this organism was introduced by Dr. McKeon in taking a sample (Appendix 18).

APPENDIX 22.

REPORT OF SEROLOGICAL AND OTHER CHARACTERISTICS OF STAPHYLOCOCCI ASSOCIATED WITH THE BUNDABERG INVESTIGATION.

Dr. F. M. Burnet, of the Walter and Eliza Hall Institute, who worked out for us the relationship of the various staphylococci isolated in these experiments, contributes the following summary of his results :—

Material.

Numerous cultures from the original bottle used by Dr. Thomson showed the same organism, and for stock a single colony was taken from one of the original serum agar slopes made on the 3rd February, 1928. This is named "Bundaberg" in the following.

From anaerobic glucose broth cultures inoculated from the original bottle a number of aureus colonies were obtained and stock culture $B.T_2$ was developed from one of these. Reasons will be given for considering this a variant of the original Bundaberg strain.

Other material was as follows :—

1. Agar slope cultures of organisms from abscesses in children's arms—
   - B.D. .. .. .. } From Dr. Richards, Rockhampton.
   - W.S. .. .. .. }
   - V.T.C. .. .. .. } From Dr. Tebbutt.
   - B.P. .. .. .. }
   - E.D. (A) .. .. .. }

   These were all of the same appearance, white with a faint creamy tinge in young cultures, as the Bundaberg staphylococcus.
2. Agar slope culture from abscess of buttock (E.D. (B)).
3. Staphylococci isolated from Dr. Thomson's water bottle (W.B.) and rainwater tank (Tank) by Dr. Tebbutt on agar slopes.
4. Several strains of staphylococci obtained from bottles of toxin-antitoxin mixture injected with air in Bundaberg, viz., F1 (albus), F3 (albus), F8 (albus), F7 (aureus).
5. Two strains isolated from bottles into which Dr. Thomson had injected air in removing samples for demonstration of his technique at Bundaberg, viz., T2.A. (albus), T2.B. (albus).

Several sera eventually pooled were made by immunizing rabbits against "Bundaberg," the final titre being approximately 1,280.

Sera were also made against strain $B.T_2$.

Agglutinating emulsions were made from all the strains available, in the usual way—growth on agar and emulsification in formalized saline to an opacity corresponding to 2,000 millions per c.cm.

These were tested with each serum with the results tabulated below. In the same table are shown the colour and the haemolytic power of the various strains on human blood agar.

Strain.		Serum "Bundaberg."	Serum $B.T_2$.	Colour.	Haemolysis on Human Blood Agar.
Bundaberg	.. .. ..	1,280	320	..	N H
	$B.T_2$	40	640	aureus ..	H
Cultures from arms of survivors	B.D.	640	40	..	N H
	E.D. (A)	640	80	..	N H
	B.P.	640	80	..	N H
	V.T.C.	640	80	..	N H
	W.S.	640	40	..	N H
Tank	.. .. ..	0	..	albus ..	N H
W.B.	.. .. ..	0	0	albus ..	
Cultures from air in T.A.T. Bottles	F1 albus	40	40	albus ..	H
	F3 albus	40	0	albus ..	H
	F8 albus	40	0	albus ..	H
	T2 A	40	0	albus ..	H
	T2 B	40	640	albus ..	N H
	F7 aureus	40	40	aureus ..	N H
	E.D. (B)	640	160	aureus ..	H

It will be seen that the cultures from the arms of the various survivors form a compact group differing in no important point amongst themselves. They are identical with the Bundaberg staphylococcus except in the one respect that they agglutinate to a consistently lower titre with the heterologous serum $B.T_2$.

Absorption experiments with the two sera and their homologous strains indicate that $B.T_2$ possesses two antigens, one in common with "Bundaberg," the other specific. This is compatible with the view that $B.T_2$ is a variant of "Bundaberg" analogous to a partly "rough" strain, although in the present absence of knowledge concerning the serological changes with staphylococcal variation no stress can be laid on this interpretation.

The serum "Bundaberg" agglutinated several typical pyogenic aureus strains from other sources to full titre, and this organism must be regarded as a serologically typical pyogenic strain as first described by Kolle and Otto, and confirmed (on the whole) by Julianelle, Hine and others. The various strains derived from air and water at Bundaberg seem to belong to the unrelated non-pyogenic strains with the possible exception of T2.B, which is closely related to the presumed variant of "Bundaberg" $B.T_2$.

The difference in the history of the strains is probably sufficient to account for the slight distinction in serological behaviour between "Bundaberg" and the strains derived from the children's arms.

The identity of all these strains is also supported by two incidental lines of evidence. They all show under appropriate conditions Müller's phenomenon on human blood agar, i.e., an appearance of punctate haemolysis in a zone extending several millimetres beyond the limits of growth of the organism. This phenomenon is rather rare amongst staphylococci, two others only of about a dozen tested showing it.

In the second place a bacteriophage was obtained weakly active against strain "Bundaberg." This was tested against a number of staphylococcal strains with the results tabulated, the number of +'s indicating the relative degree of lysis on agar.

"Bundaberg"	.. ..	+++	E.D. (Buttock) .. .. —
$B.T_2$. —	.. ..	—	F_7. aureus .. .. —
B.D.	.. ..	++	Six staphylococcal strains not
B.P.	.. ..	++	connected with the Bunda-
W.S.	.. ..	++	berg investigation. All —
V.T.C.	.. ..	+++	
E.D. (A)	.. ..	+++	

In all cases where lysis occurred it was incomplete and was followed by secondary growth. Staphylococcal bacteriophages fall into two types, one type is active against almost all varieties of staphylococci with the exception of specifically resistant variants, the other is practically confined to the homologous strain (d'Herelle, Gratia, Callow and others). The sensitivity of all the arm cultures and these only to the phage active against "Bundaberg" is, therefore, very strong evidence of their identity with this strain.

In regard to the strain E.D. (buttock), its characteristics (aureus, haemolytic, not sensitive to phage) are sufficient to separate it from the "Bundaberg" group despite its practical serological identity. It resembles in all respects a normal pyogenic aureus and is serologically distinct from the aureus variant obtained from "Bundaberg."

Later work has shown that in its agglutination and absorption reactions its sensitivity to bacteriophage and its reactions on blood agar the Bundaberg strain is identical with Hine's Type II. (*Lancet*, 1922), strain 1,393 of the national collection of type cultures.

Conclusions.

The organism "Bundaberg" obtained from the implicated bottle is identical with the staphylococci recovered from the children's arms in the cases of B.D., W.S., B.P., E.D. and V.T.C.

None of the organisms obtained by injection of air into toxin-antitoxin bottles (or from the water supply) at Bundaberg are of this type.

The staphylococcus obtained from the lesion in the buttock (E.D. (B)) is also not of the type.

APPENDIX 23.

THE PATHOGENICITY OF THE BUNDABERG STAPHYLOCOCCUS TO LABORATORY ANIMALS.

The experiments detailed in Appendix 18 do not suggest that this organism is, with the single exception of the rabbit, highly pathogenic for the ordinary laboratory animals. A large number of experiments with guinea pigs, and a few with mice, rabbits and monkeys have been concerned with the pathogenicity of the staphylococcus when grown in toxin-antitoxin mixture (Appendix 26).

In this place we have gathered together a few of the results of inoculation of cultures and emulsions from various media which indicate the somewhat variable pathogenicity of the organism for rabbits, and its low pathogenicity for monkeys, guinea pigs, and mice.

Subcutaneous and intramuscular injections in these animals do not produce fatal results. These can only be obtained by intravenous or intraperitoneal inoculation.

In mice of about 20 grams weight inoculated intraperitoneally with a saline emulsion from a 24-hours agar slope culture, 5,000 million organisms kill in from six to eight hours, 500 million in twenty hours, while with doses of 50 millions the animals survive.

In guinea pigs of 250 to 300 grams weight, 500 to 1,000 million organisms cause a small local abscess when injected subcutaneously. There is also a temporary decline in body weight. Doses of 200 million organisms injected intravenously kill on about the fourth day. A fatal result is produced in six days by injecting intravenously 0.5 c.cm. of a twenty-hour broth culture while the same culture in twice the dose injected intramuscularly fails to kill and causes only local abscesses.

In rabbits weighing approximately 1 kilo., 200 and 400 million organisms when injected intravenously killed on the second and sixth day, and 10 million organisms killed one of two animals on the tenth day. Five hundred and 1,000 million staphylococci injected subcutaneously caused only a small local lesion and the same doses given intramuscularly gave only local effects with small loss of weight. Comparing intravenous and intramuscular injections with a twenty-hour broth culture, doses of 1.0 c.cm. by the latter route caused only local lesions and loss of weight, while half this dose given intravenously killed on the third day. In these late deaths the usual pyaemic lesions in the kidneys were found at autopsy.

Intravenous injections of broth cultures of the staphylococcus were also made into a number of young monkeys.

Monkeys 5, 6, and 7 had earlier received subcutaneous injections of cultures of the staphylococcus in toxin-antitoxin mixture. The results of these injections are described in Appendix 26.

Monkey 5, which weighed 2·1 kilos, received 1.0 c.cm. of a 24 hours' culture which had been inoculated with one platinum loopful of a standard emulsion from an agar slope, which was itself a subculture from a culture of the staphylococcus grown in a mixture of monkey blood and toxin-antitoxin. No effect was apparent in thirteen days and the weight was then 2·5 kilos.

Monkey 6, which weighed 2·27 kilos, received 2·0 c.cm. of a 24-hour broth culture inoculated direct from defibrinated monkey blood, in which the staphylococcus had grown for three days, after passage through decreasing dilutions of monkey blood with toxin-antitoxin mixture. A slight rise in temperature was observed next day, but no obvious illness resulted.

After nine days the weight was found to be 2·13 kilos. The lymph glands in the groin were enlarged though the local lesions resulting from the previous subcutaneous inoculations had quite disappeared.

Monkey 7, which weighed 2·35 kilos, received 2·0 c.cm. of the same broth culture as monkeys 6 and 10, but after 48 hours incubation. A slight rise of temperature also occurred next day, but no obvious illness resulted for eight days when his weight was found to be 2·38 kilos and he appeared well. Two days later he went off his food. On the eleventh day he was obviously ill, eating very little, temperature only 36° C. He was found dead but still warm the

next morning. He had not had diarrhœa. Weight 1·9 kilos. Autopsy revealed a remarkable picture of acute generalized staphylococcal infection. There were haemorrhages into the skin and serous membrane and small vegetations on the mitral valve. The spleen was enlarged and firm and showed recent firm infarcts of large size. The liver was congested but showed no focal lesions. The kidneys showed haemorrhages and one recent infarct and were much swollen. The retroperitoneal and mesenteric lymph glands were markedly swollen and of dark colour. Cultures gave staphylococci resembling "Bundaberg" from heart blood, liver, spleen, lymph glands (several), and kidney all in pure culture.

The histological examination of the liver shows congestion of the sinusoids, central veins and portal branches, accumulations of small and large mononuclear cells in some of the portal tracts, and occasional small areas of focal necrosis in or near portal tracts.

In the spleen there is much engorgement of the pulp, and relatively small malpighian bodies, many of which show central necrosis, sometimes involving thrombosis of the arteriole.

All the glands examined show haemolymph characters. They are extremely engorged and oedematous with dilatation especially of the medullary sinuses which contain abundant large phagocytic endothelial cells stuffed with red corpuscles. A large number of the lymph follicles have necrotic centres which, like those seen in the liver and spleen, are mostly in process of absorption.

In many points there is a close resemblance in the condition of these glands to some of those examined from the children at Bundaberg, and the appearances observed suggest the possibility that some of these also may be of haemolymph type, though the difficulty or impossibility of recognizing red cells in sections of engorged vessels in these cases allows no security of opinion.

Gram's stain reveals abundant staphylococci distributed everywhere through the tissues examined. They are seen in the blood stream, occasionally plugging small vessels in the glands and spleen, in phagocytes, in the swollen Küpffer cells and even in the liver cells themselves. It is notable that their presence does not appear at any point to excite any focal reaction and nothing in the nature of abscess formation is seen. Polymorphs are not a conspicuous feature of any of the sections.

Monkey 10, weighing 2·75 kilos, received 1·0 c.cm. of the same broth culture (24 hours) as Monkey 5. The temperature response following the injection may be regarded as typical in this series of experiments. Before inoculation the rectal temperatures of all the animals lay between 38·5° C. to 38·75° C. On the second and third days the temperatures in Monkey 10 were 38·75° C. and 39° C. and the animal was sulky. On the sixth and the following three days the temperatures were 39·2° C., 38·9° C., 38·9° C. and 38° C., and the animal looked well and was active and eating well. Its weight on the fourteenth day was 2·6 kilos. There were then enlarged glands in the groin particularly on the right side, but no other signs or symptoms of illness.

Monkey 11, weighing 1·6 kilos, temperature 38·3° C., received 2·0 c.cm. of the same broth culture (48 hours) as Monkey 7. This animal was already underweight and sullen. On the second day the temperature was 38·1° C., on the third 38·6° C., on the fourth 37·8° C. It developed diarrhoea and on the seventh day the temperature was still lower. It refused food and was moribund on the ninth day. No abscesses were discovered at autopsy and no lesions of the internal organs other than enlargement of the mesenteric lymph glands. Cultures from the heart blood and pericardial fluid were sterile. The cultures from the spleen when plated grew a few colonies resembling the Bundaberg stphylococcus. Death was apparently due to enterocolitis.

Monkeys 7 and 11 were the only animals in this series which succumbed, and of these Monkey 7 alone showed a generalized infection. Monkey 11 received the same intravenous injection but not the preliminary subcutaneous ones, and died within the period of development of the disease in Monkey 7, possibly from an intercurrent bowel affection. There is a possibility that the previous subcutaneous injection of a four-day culture of the staphylococcous in toxin-antitoxin mixture may have determined the fatal issue, but it seems more probable that the important factors were the larger number of organisms later introduced (48 hours culture) and the individual susceptibility of the animal in question.

All the experiments recorded above were carried out two to three months after the organism had been isolated, without passage through any animals in the meanwhile. Some earlier experiments with rabbits recorded in the table below give indications of much greater pathogenicity :—

EFFECT OF INTRAVENOUS INJECTION OF THE BUNDABERG ORGANISM INTO RABBITS.

—	Body weight in kilos.	Dose.	Result.
1 ..	1·9	0·1 c.cm. glucose brain broth culture eleven hours (inoculated from 78a B* 24 hours after inoculation from B.T.AT.)	Died with convulsions in eleven hours P.M. Hæmorrhages in lumbar muscles, meningeal congestion, staphylococci in pure culture from heart blood.
2 ..	1·5	1·0 c.cm. 24 hours, glucose brain broth heart blood culture from 1	Dead in 22 hours. Necrotic lesions in liver, pericarditis, infarction in both kidneys, hæmorrhages in ribs at costochondral junction.
3 ..	1·4	0·5 c.cm. of same	Dead in 23 hours. Infarcts in kidneys—necrotic lesions in liver.
4 ..	1·45	0·2 c.cm. 6-hours glucose brain broth culture of heart blood 2	Survived.
5 ..	1·3	0·5 c.cm. of same	Survived
6 ..	1·4	0·2 c.cm. of 27 hours brain broth culture of heart blood of 2	Death in thirteen hours with convulsions, punctate necrosis in liver, infarct in right kidney, staphylococcus isolated in pure culture from heart blood.
7 ..	1·5	0·25 c.cm. glucose brain broth (sub of original culture)	Died in five days. Septic infarcts in kidneys and necrotic areas in liver.
8 ..	1·2	0·25 c.cm. of serum from heart blood taken by intracardiac puncture from 7 (on third day) and incubated 24 hours	Died in 24 hours, no lesions.
9 ..	1·3	0·25 c.cm. 48-hours culture of heart blood of 7 in glucose brain broth	Survived.

*78a B=bottle of batch 78A inoculated from the original Bundaberg bottle (B.T.AT.) and incubated at room temperature (Appendix 18).

For the ordinary laboratory animals this staphylococcus is of about average virulence. Its virulence for rabbits is somewhat variable. The first sub-culture from the Bundaberg bottle showed surprising virulence and killed a large rabbit so quickly as to give no time for the production of any obvious lesions.

Two of these animals which died rapidly, convulsed continuously before death. This symptom was never seen in the animals which died after longer periods with a less acute illness.

APPENDIX 24.

PRODUCTION OF A SKIN-REACTING TOXIN FROM THE BUNDABERG STAPHYLOCOCCUS.

It is known that filtrates from staphylococcal cultures may produce positive skin reactions when injected intradermally in susceptible human individuals. This offered a suggestion for an attempt to demonstrate the formation of an active toxin by the Bundaberg staphylococcus, and Dr. F. M. Burnet has kindly carried out experiments for us of which a brief résumé is given here.

If a staphylococcal toxin played any important part in causing the Bundaberg deaths, it must have been produced within a few hours of the introduction of the organisms into the body. For this reason the tests were all made with filtrates from young cultures seven to eight hours old. Preliminary experiments were done with cultures in a mixture of toxin-antitoxin and human blood. In susceptible individuals a positive skin test was obtained with 1 in 500 or 1 in 1,000 dilutions of a filtrate from such a culture. The reaction showed as an area of erythema about 2 cm. in diameter with occasional slight subjacent swelling in all respects similar to a positive Schick or Dick test. Later work showed that similar reactions were obtainable when the cultures were grown in human blood broth and this medium was used for most of the experiments.

5 c.cm. of freshly-drawn human blood was added to 10 c.cm. of citrated broth. The tube was heavily inoculated from a young agar culture of the required staphylococcal strain and incubated at 37° for seven or eight hours. The supernatant fluid was then pipetted off from the deposited corpuscles and after dilution with saline (1 in 3) was passed through a Seitz filter. The filtrate was tested for sterility and diluted to 1 in 500 as required. In addition to the Bundaberg strain another staphylococcus (S.I.) was used. This was a typical pyogenic aureus strain derived from a human skin lesion.

A mixed group of individuals was tested with these filtrates by the injection of 0.2 c.cm. of 1 in 500 dilutions with the following results :—

—	S. I.	Bundaberg.
Number tested	36	44
Number positive	16	20
Number negative	17	18
Number doubtful	3	6

In each instance about half the individuals tested were sensitive to the filtrate at this dilution.

The skin-reacting substances present in filtrates from the two organisms seem to be distinct one from the other. The degree of reaction noted in each of the individuals who received both filtrates is shown in the following table :—

Subject.	B.	S. I.	Subject.	B.	S. I.
C. K.	—	++	D. A.	++	++
F. B.	++	++	G. B.	+	++
B. S.	++	++	T. C.	—	—
H. W.	+	++	T. D.	+	+
E. K.	—	++	A. H.	++	—
N. A.	—	±	S. L.	—	—
K. A.	—	—	C. O.	++	—
K. B.	+	—	F. P.	++	+
J. C.	++	—	G. S.	++	—
B. D.	—	—	J. B.	±	++ (Subcutaneous)
R. G.	+	—	L. C.	—	++ (Subcutaneous)
A. J.	—	—	J. H.	—	— (Subcutaneous)
G. L.	±	—	M. J.	+	++ (Subcutaneous)
V. M.	±	±	M. R.	+	+ (Subcutaneous)
G. M.	—	—	N. S.	—	— (Subcutaneous)
R. S.	±	—	E. H.	+	— (Subcutaneous)
F. T.	—	±	E. M.	++	+ (Subcutaneous)
L. A.	—	+	R. W.	+	++ (Subcutaneous)

++ denotes a reaction 1 inch or more in diameter.
+ denotes a reaction ½ inch to 1 inch in diameter.
± denotes a reaction less than ½ inch in diameter.
— denotes no reaction.

It will be seen that occasionally either filtrate may give a strong reaction while the other is completely inert.

Filtrates from staphylococcal cultures in plain broth grown for the same length of time are definitely less active than those from blood broth cultures although some skin-reacting substance is produced. It seemed possible that differences in the amount of active agent produced might be found according to whether the blood used in the medium were derived from susceptible or non-susceptible individuals. The "Bundaberg" staphylococcus was, therefore, grown in citrated broth—

(*a*) with blood from a positively reacting individual,
(*b*) from a non-reacting subject,
(*c*) without addition of blood.

Filtrates were tested on a susceptible subject with the following results :—

—	Dilution.	Diameter of Reaction.
Filtrate made with "susceptible" blood	1 in 1,000	2·5 cm.
	1 in 2,000	Nil
Filtrate made with "non-susceptible" blood	1 in 1,000	2·2 cm.
	1 in 2,000	2·2 cm.
Filtrate from culture without blood	1 in 1,000	Nil
	1 in 2,000	Nil

If anything, the "non-susceptible" blood produces more of the skin-reacting substance, but it is doubtful if the difference is significant.

We have further attempted in two of the workers in this Institute, F.B. and B.S., to ascertain whether the serum from N.W. (Case 8) who had no symptoms following the injection on 27th January, could protect in sensitive individuals in a dilution of 1 in 20 against 1 in 1,000 and 1 in 400 of the toxin (filtrate of Bundaberg staphylococcus blood broth cultures). The serum itself in this dilution produced a very slight reaction which is not surprising in view of its age and the possibility of contamination. The dilution was of course filtered before use. As controls in this experiment we injected 1 in 1,000 of the toxin alone and 1 in 1,000 and 1 in 400 of the toxin mixed with a 1 in 10 dilution of the sera of R.G. and J.C. (both known skin reactors).

—	F. B.	B. S.
1/1,000 B. Filtrate	++ (2·0 cm.)	++ (2·2 cm.)
1/1,000 B. Filtrate 1/20 N.W.	+ (1·5 cm.)	+ (vague)
1/400 B. Filtrate 1/20 N.W.	+++ (3·1 cm.)	++ (2·5 cm.)
1/1,000 B. Filtrate 1/10 J.C.	++ (2·2 cm.)	++
1/1,000 B. Filtrate 1/10 R.G.	+ (1·5 cm.)	± (2·2 cm.)

These preliminary observations are too few for any stress to be laid upon their interpretation. The Bundaberg staphylococcus grown under appropriate circumstances can produce a substance whose activity is shown in specific skin reactions in a proportion of human individuals. It may be that these reactions indicate differences in susceptibility to the general pathogenic activity of the organism. This interpretation would be analogous to the current view of the relationship between the Dick test and the variation of pathogenicity of the streptococcus of scarlet fever in human individuals. A further point of resemblance is afforded by the absence of any toxic effects when skin-reacting filtrates are injected in doses of 5 c.cm. (undiluted) into laboratory animals (monkey, guinea pig and rabbit).

If the Bundaberg staphylococcus were responsible for the illness and deaths which followed its subcutaneous injection on 27th January, these observations may be held to suggest a partial explanation of the failure of some of the children to show any symptoms. It is obvious that the crucial experiment of ascertaining whether the survivors at Bundaberg are or are not sensitive to the skin-reacting substance cannot be carried out.

APPENDIX 25.

THE EFFECT OF THE BUNDABERG STAPHYLOCOCCUS ON TOXIN AND ANTITOXIN SEPARATELY.

For this purpose it was decided to use toxin diluted to about the same concentration as in Batch 86, to which normal sterile horse serum was added so as to make the protein content of the mixture about equal to that of the toxin-antitoxin mixture. Similarly antitoxin was made up containing approximately one unit per c.cm. with sugar free broth to take the place of toxin.

The toxin used was pooled 21A and 21D of the Commonwealth Serum Laboratories. The antitoxic serum used was also prepared by the Commonwealth Serum Laboratories and consisted of mixed batches of returned sera similar to those used in the preparation of Batch 86.

It was first of all necessary to determine the approximate L + dose of the toxin and the titre of the antitoxin.

For the determination of the L + dose a number of guinea pigs weighing 250 grams were inoculated subcutaneously with mixtures consisting of varying amounts of toxin with 1·0 unit of standard antitoxin. The mixtures were made up to a volume of 4·0 c.cm. with saline and allowed to stand one hour before injection.

The results of these tests are shown in Table 1.

TABLE 1.

DETERMINATION OF L + DOSE OF TOXIN.

Number of Animals.	Dose of Toxin.	Result.
	c.cm.	
One	·200	Infiltration—loss of weight until seventh day. Survived.
One	·225	Infiltration—loss of weight until seventh day. Survived.
Five	·250	Died 64 hours, 87 hours, 94 hours, 94 hours and 101 hours.
Two	·260	Died 66 and 67 hours.
Two	·275	Died 77 and 72 hours.
One	·300	Died 44 to 64 hours.
One	·325	Died 44 to 64 hours.
One	·350	Died 42 hours.

The L + dose was, therefore, about ·250 c.cm. for this toxin.

This dose of toxin was now put up with varying doses of 1 in 1,000 dilution of the antitoxic serum to be used. The mixtures were made up to 4·0 c.cm. with saline and allowed to stand one hour before injection into guineapigs of 250 grams weight. The results are shown in Table 2.

TABLE 2.

TITRATION OF ANTITOXIN.

Number of Animals.	Dose of 1 in 1000 Serum.	Result.
	c.cm.	
Two	0·71	Died 54 to 63 hours.
Two	0·74	Died 58 to 72 hours.
Two	0·77	Died 58 to 65 hours.
Two	0·80	Died 60 to 87 hours.
One	0·85	Died seventh day.

We, therefore, regarded this antitoxin as containing approximately 1,250 units per c.cm.

The mixtures were, however, made up before the results of this last titration were complete and were as follows:—

A. Toxin Mixture—

Toxin	20 c.cm.
Horse serum 1 in 20 in saline	1·5 c.cm.
Saline	80 c.cm.

B. Antitoxin Mixture—

Sugar free broth	10 c.cm.
Antitoxin 1 in 20 in saline	1·5 c.cm.
Saline	90 c.cm.

In spite of the too small proportion of antitoxin in B, when 1·5 c.cm. was added to an equal quantity of A. and the mixture injected subcutaneously into a guineapig, the animal showed no infiltration and gained weight steadily.

The mixtures were made up with full aseptic precautions and proved to be sterile in bulk. Their P.H. was approximately 7.0.

They were now bottled in sterile amber glass bottles (obtained from used samples of Batch 86) containing approximately 10 c.cm. each and covered with rubber caps from the same source. They were tested for sterility separately by inoculation of 0·5 c.cm. into 10 c.cm. of broth, and all but one or two bottles were found to be uncontaminated.

They were now inoculated with the Bundaberg staphylococcus and incubated at 80° F. The dose inoculated was estimated by plating to be 7,000 cocci. Growth took place in all the bottles. (See Appendix 26.)

Each day in the earlier days of the experiment and later at longer intervals the titre of toxin and of antitoxin was determined, and at the beginning and end of the experiment the original bulk was similarly tested. In the case of antitoxin a further control was provided by incubating an uninoculated rubber-capped bottle seven days at 80° F. Antitoxin was titrated by the intradermic method using 0·1 c.cm. of the following range of dilutions of the mixture—1 in 12·5, 1 in 16·6, 1 in 25, 1 in 33·3 and 1 in 50, corresponding to ·008, ·006, ·004, ·003 and ·002 unit of antitoxin if the mixture had contained 1·0 unit of antitoxin per c.cm. An equal volume of standard toxin solution was added to each dilution in a dose of ·0009 c.cm. in each 0·1 c.cm. Protection experiments were also carried out using some or all of the following dilutions of B—1 in 80, 1 in 70, 1 in 60, 1 in 50, 1 in 40, 1 in 30, 1 in 25 and 1 in 20. One c.cm. of each of these was mixed with the C.L.D. of toxin, 0·01 c.cm. in 1 c.cm. of saline and the mixture after standing an hour was injected subcutaneously into guineapigs weighing between 240 and 260 grams. Animals which survived were discarded on the twentieth day or later.

Toxin was titrated using the toxin mixture A. in dilutions of 1 in 24, 1 in 22, 1 in 20 and 1 in 18 for intradermic injections of 0·1 c.cm. mixed with 0·1 c.cm. of saline containing ·002 unit of standard antitoxin. The total volume of the injections in the intradermic tests was in every case 0·2 c.cm.

The content of toxin in A was also checked by injecting 1 c.cm. of 1 in 20 of the mixture equivalent to 0·01 c.cm. of toxin if no deterioration had occurred, and occasionally in a dilution of 1 in 16·6 and 1 in 14·3 to indicate the extent of any loss of activity.

The results of these experiments are set out in Tables 3. and 4. Judged by the intradermal test no appreciable destruction of toxin occurred during eighteen days incubation. The guinea-pigs injected with what should have been equivalent to the C.L.D. of toxin did not all die, so that it is possible that a small loss of activity resulted from the growth of the staphylococcus. On the eighteenth day 1 c.cm. of 1 in 20 of the bottle containing the staphylococcus killed both animals into which it was injected on the third day.

Mr. Holden had been able to recover a trace of iodine (Appendix 19) in the washings from the interior of the Bundaberg bottle. This amounted to 0.003 m.gms. in 0·075 c.cm. of mixture left in the bottle (estimated by weighing). This amount corresponded to 0·04 m.gms. per c.cm. of mixture and had evidently been introduced into the bottle by successive punctures through the rubber cap. We attempted to ascertain whether the presence of iodine had any effect. The staphylococcus inoculated into a bottle of A. containing iodine in half the concentration observed by Mr. Holden grew satisfactorily and the intradermal tests indicated a slight but definite decrease in the activity of the toxin.

The effects of the growth of the Bundaberg staphylococcus on antitoxin were somewhat greater than those observed in the case of toxin. The intradermal tests indicated a decrease of about one-third in antitoxic value after eighteen days.

The protection experiments confirmed this finding though they suggest that the decrease is about 1/5 and that some falling off of antitoxic activity takes place in this dilution at 5°C. even in the absence of bacterial contamination. 1.0 c.cm. of a dilution of 1 in 50 of the original mixture protected against the C.L.D. of toxin. After eighteen days at 5°C. 1 c.cm. of 1 in 40 was required. The bottle incubated seven days was unfortunately contaminated though it was still clear and grew only a few colonies on plating. This control was, therefore, abandoned. Of the material in which the Bundaberg organism was growing, 1 c.cm. of 1 in 40 was necessary after eighteen days incubation, for protection against the C.L.D. of toxin.

It cannot be inferred from these experiments that the Bundaberg organism is capable of destroying antitoxin preferentially in toxin-antitoxin mixtures. In our experiments (*vide* Appendix 26) we have been unable to obtain any evidence that such is the case.

TABLE 3.

TITRATION OF TOXIN ON SUCCESSIVE DAYS AFTER INOCULATION.

—	Ln/500 Dose Intradermic.	Inoculation of Mixture into Guinea Pigs Subcutaneously.		
		No. of Animals.	Dose.	Results.
Original mixture	·1 c.cm. of 1 in 22	2	1 c.cm. .. 1 in 20 ..	Died on the third and fifth days.
After 49½ hours incubation ..	·1 c.cm. of 1 in 22	2	1 c.cm. .. 1 in 20 ..	One died on the sixth day, the other survived gaining weight by eleventh day.
After 75 hours	·1 c.cm. of 1 in 22	2	1 c.cm. .. 1 in 16·6	Both died on the third day.
After 99 hours	·1 c.cm. of 1 in 22	2	1 c.cm. .. 1 in 16·6	One died on the fifth day, the other regained body weight by the fifteenth day.
After 123 hours	·1 c.cm. of 1 in 22			
After 7 days	·1 c.cm. of 1 in 22	2	1 c.cm. .. 1 in 20 ..	One died on the fifth day and one regained body weight by twelfth day
After 12 days	·1 c.cm. of 1 in 22	2	1 c.cm. .. 1 in 20 ..	Died on the third and twenty-sixth days..
		2	1 c.cm. .. 1 in 16·6	Died on the third and fifth days.
After 18 days	·1 c.cm. of 1 in 22 (·1 c.cm. of 1 in 24 still nearly necrosis level)	2	1 c.cm. .. 1 in 20 ..	Died on the fourth and seventh days.
		2	1 c.cm. .. 1 in 16·6	Died on the third and sixth days.
		2	1 c.cm. .. 1 in 14·3	Died on the third and fourth days.
Original mixture at end of experiment	·1 c.cm. of 1 in 22 (·1 c.cm. of 1 in 24 nearly necrosis level)	2	1 c.cm. .. 1 in 20 ..	Both dead on third day.
		2	1 c.cm. .. 1 in 16·6	Died on third and fourth days.
		2	1 c.cm. .. 1 in 14·3	Both dead on third day.
Original mixture + Iodine incubated 8 days	·1 c.cm. of 1 in 20	2	1 c.cm. of 1 in 20	One died on fourth day and one regained weight by the thirteenth day.
		2	1 c.cm. of 1 in 16·6	Both died on the fourth day.
		2	1 c.cm. of 1 in 14·3	Both died on the fourth day.
Original mixture + Iodine, incubated. 12 days	·1 c.cm. of 1 in 22	2	1 c.cm. .. 1 in 20 ..	One died on eighth day the other regained body weight by 13th day.

TABLE 4.

TITRATION OF ANTITOXIN ON SUCCESSIVE DAYS AFTER INOCULATION.

—	Intradermal Titration. 1/500 Unit A.T. in 0·1 c.cm. of—	Injection of 1 c.cm. of varying Dilutions of Antitoxin Mixture with 1 c.cm. containing .01 Toxin.		
		No. of Animals.	Dilution of A.T. Mixture.	Results.
Original mixture before inoculation	1 in 50	3	1 in 50 ..	Extensive infiltration and later ulceration—original weight regained by fifteenth, seventeenth, and twenty-third days.
			1 in 60 ..	One died on the seventh, one on the twenty-sixth day, and one had regained its original weight by the twenty-first day.
		3	1 in 70 ..	Died on fourth, sixth and eighht days.
		3	1 in 80 ..	One died on the ninth, and two on seventh day.

TABLE 4.—TITRATION OF ANTITOXIN ON SUCCESSIVE DAYS AFTER INOCULATION—*continued.*

—	Intradermal Titration. 1/500 Unit A.T. in 0.1 c.cm. of—	Injection of 1 c.cm. of varying Dilutions of Antitoxin Mixture with 1 c.cm. containing .01 Toxin.		
		No. of Animals.	Dilution of A.T. Mixture.	Results.
After 49½ hours	1 in 50	2	1 in 25 ..	No infiltration, steady gain in weight.
		2	1 in 50 ..	Extensive infiltration and necrosis. Weight regained by twenty-first day.
		2	1 in 60 ..	Both died on seventh day.
		2	1 in 70 ..	Both died on ninth day.
		2	1 in 80 ..	Died on seventh and ninth days.
After 75 hours	1 in 50 .. .. (1 in 33·3 gives more marked reaction than earlier)	2	1 in 20 ..	No infiltration, steady gain in weight.
		2	1 in 30 ..	Slight infiltration, no loss of weight
		2	1 in 40 ..	Infiltration, loss of weight—regained by nineteenth day.
		2	1 in 50 ..	Infiltration, loss of weight—not regained by twenty-first day.
		2	1 in 60 ..	Died on fourth and seventh days
After 99 hours	1 in 50 .. .. (1 in 33·3 reaction nearly necrosis level)	2	1 in 40 ..	Infiltration, necrosis, regained weight by nineteenth day.
		2	1 in 50 ..	Infiltration, necrosis, regained weight by seventeenth day.
		2	1 in 60 ..	One died on the seventh and one regained body weight by twentieth day.
		2	1 in 70 ..	Died on sixth and eighth days
After 123 hours	1 in 50 .. .. (1 in 33·3 nearly necrosis level. Reaction with 1 in 25 persists 4 days	2	1 in 40 ..	Extensive infiltration and necrosis, one regained weight by eighteenth day.
		2	1 in 50 ..	One died on the seventh and the other had not regained weight by eighteenth day. Paralysis of left hind limb.
		2	1 in 60 ..	One died on sixth and the other regained weight by fourteenth day.
After 7 days	1 in 50 .. .. Reactions as after 123 hours	2	1 in 25 ..	Slight infiltration. Slight loss of weight in one only.
		2	1 in 40 ..	Infiltration, necrosis. Body weight regained by sixteenth day.
		2	1 in 50 ..	One died on the eighth and the other regained body weight by sixteenth day.
		2	1 in 60 ..	Both died on fifth day.
After 12 days	Between 1 in 50 and 1 in 33·3 ..	2	1 in 20 ..	Definite slight infiltration but no loss of weight.
		2	1 in 30 ..	Infiltration, no loss of weight
		2	1 in 40 ..	Infiltration, necrosis—body weight regained by ninth day.
		2	1 in 50 ..	One died fourth day and one had infiltration and necrosis and regained weight on seventh day.
		2	1 in 60 ..	Both died on twenty-fourth day.
After 18 days	1 in 33·3 ..	2	1 in 20 ..	No infiltration. Steady gain in weight.
		2	1 in 30 ..	Infiltration. Steady gain in weight
		2	1 in 40 ..	Infiltration and necrosis — body weight regained by fifth and twelfth days.
		2	1 in 50 ..	Died on the seventh and eighth days.
		2	1 in 60 ..	Died on the fourth and eighth days.
Control bottle uninoculated seven days incubated	1 in 50 .. .. (1 in 33·3 gives good reaction nearly to necrosis level)	2	1 in 20 ..	Very slight infiltration. Slight loss of weight in one only.
		2	1 in 30 ..	Infiltration. Steady gain in body weight.
		2	1 in 40 ..	Infiltration—body weight regained by seventh day.
		2	1 in 50 ..	Infiltration, necrosis, body weight regained in one by eighth day but in the other was not regained by the eighteenth day.
		2	1 in 60 ..	Died fourth day and seventh day.

TABLE 4.—TITRATION OF ANTITOXIN ON SUCCESSIVE DAYS AFTER INOCULATION—*continued.*

—	Intradermal Titration. 1/500 Unit A.T. in 0·1 c.cm. of—	Injection of 1 c.c. of varying Dilutions of Antitoxin Mixture with 1 c.cm. containing .01 Toxin		
		No. of Animals.	Dilution of A.T. Mixture.	Results.
Control Stock at end of Experiment	1 in 50	2	1 in 30 ..	Infiltration—one regained original weight on fifth and the other on twelfth day.
		2	1 in 40 ..	Infiltration and necrosis. Both regained weight by twelfth day.
		2	1 in 50 ..	One died on the twenty-sixth day, the other regained weight by twelfth day.
		2	1 in 60 ..	One died on the seventh day and the other had not regained weight by the twelfth day.
		2	1 in 70 ..	One only regained weight by the twelfth day.

APPENDIX 26.

ON THE CULTIVATION OF STAPHYLOCOCCUS IN TOXIN-ANTITOXIN, ON THE POSSIBILITY OF DISSOCIATION OF TOXIN BY THIS METHOD, AND ON THE PATHOGENICITY OF STAPHYLOCOCCI GROWN IN THIS MEDIUM.

Toxin-antitoxin as prepared by the Commonwealth Serum Laboratories cannot compare with plain broth as a culture medium for staphylococci. The particular batch (No. 86) with which we are mainly concerned was made up to contain in 1 c.cm. 80 per cent. of an L + dose of toxin and one unit of antitoxin. The L + dose of the toxin used was 0.325 c.cm. and the titre of the antitoxin 1,350 units per c.cm. Therefore, each c.cm. of toxin-antitoxin should contain—(1) .260 c.cm. of toxin (i.e. broth in which first colon bacilli, to render sugar free, and then diphtheria bacilli have been grown), (2) .00073 c.cm. of antitoxin (i.e. horse serum globulins) and (3) normal saline made up to 1 c.cm. Regarded as a nutrient medium it consists of one quarter strength broth, in which already two types of bacteria have been cultivated, and a very minute amount of serum globulin.

Immediately after the cultivation of the staphylococcus from the Bundaberg bottle subcultures were made into other bottles and after varying periods at room temperatures or at 37° C. animals were inoculated. From one bottle doses of 1.0 c.cm. given subcutaneously after one, two three and nine days at room temperature produced no obvious effects in guinea pigs, but it was noted that whilst the first three animals gained 104, 122 and 92 grams in weight in twenty-two, twenty-six and twenty-five days respectively, the fourth animal, inoculated with the nine days' growth, only gained seven grams in a corresponding time.

From another inoculated bottle kept at room temperature, subcutaneous injections of 1.0 c.cm. were given with the following results :—

Time after Contamination.	Results in Guinea Pigs.	Weight before Inoculation.	Weight afterwards—Days in Brackets.
4 hours	Nil	249	332 (20)
23 hours	Nil	243	358 (23)
2 days	Late ulceration	250	392 (26)
3 days	Nil	234	324 (26)
4 days	Late ulceration	230	327 (25)
6 days	Firm induration	255	274 (24)
9 days	..	241	242 (21)

The first five animals inoculated on the first four days gained on the average 105 grams in weight in 24 days, whilst the animal inoculated on the sixth day which early showed a firm indurated area in the abdominal wall only gained 19 grams in 24 days, and the animal injected on the ninth day showed no gain in weight after 21 days.

Another series of animals were inoculated subcutaneously with doses from a bottle of Batch 78A (toxin-antitoxin) infected directly from the original Bundaberg bottle and incubated for 24 hours at 37° C. 1.0 c.cm. injected into a guinea pig produced a small local abscess healing to a clean scar by the tenth day and the animal gained only 27 grams in nineteen days. 0.5 c.cm. also produced late ulceration but the animal gained 67 grams in nineteen days. 0.2 and 0.1 c.cm. produced no obvious lesions and the animals gained 63 and 60 grams respectively in nineteen days. A rabbit showed only a slight local lesion after a subcutaneous inoculation of 0.5 c.cm. and gained well in weight. Four mice received an intraperitoneal inoculation of 0.2 c.cm. They appeared to be very sick after five hours with neurotoxic symptoms but seemed well on the following day. One died after six days with multiple abscesses in both kidneys.

Another bottle of Batch 78A inoculated direct from the fatal bottle was kept at room temperature. Of two mice inoculated intraperitoneally after 24 hours with 0.1 c.cm. one was moribund in four hours and the other survived. After 48 hours four mice received the same dose and one only died in four days.

Eleven guinea pigs also were inoculated from two bottles of Batch 78A infected directly from the fatal bottle. Doses ranging from 1.0 to 0.1 c.cm. and injected subcutaneously after varying periods up to four days produced no deaths and scarcely any local effects. The four days' growth, however, produced some necrosis of the skin.

The results of these early animal experiments suggested to us that a more detailed study should be undertaken in which animals would be inoculated daily with the same dose, and at the same time the number of cocci enumerated.

Graph 1 M.S.C. 4. Periodic enumeration of the Bundaberg Staphylococcus growing in T.A.T. at 30° Fah. (26·6° C.) and the results of subcutaneous inoculations of guinea pigs with 0·5 c.cm. (O. Tr. +. ++ and +++ indicate increasing degrees of local lesion.)

Graph 1 shows the counts obtained at intervals in a 10 c.cm. rubber-capped bottle of toxin-antitoxin (Batch 86) marked M.S.C. 4 and also the local effects of subcutaneous inoculation in the abdominal wall of guinea pigs.

The rubber cap was sterilized by filling the cup-like depression in it with methylated spirit, most of which after two minutes was gently shaken out and the remainder flamed. With a sterile 1 c.cm. tuberculin syringe 0.1 c.cm. of a very dilute saline emulsion of the staphylococcus was injected through the rubber cap and the bottle inverted several times to mix evenly. The same amount was well mixed with melted agar and the latter plated. Counts made after two days at 37° C. show that the inoculum was approximately 550 organisms per c.cm. of toxin-antitoxin. This bottle was incubated at 80° F. (26.6° C.) and from the second to the fourteenth day, excepting the tenth and eleventh days, the bottle was taken out on to the bench for several hours, the contents gently agitated, and 0.6 c.cm. withdrawn with a syringe. 0.1 c.cm. was mixed with agar and plated or known dilutions of it were made and plated. At the same time the remaining 0.5 c.cm. was inoculated subcutaneously into a guinea pig. As may be seen from the graph, animals were inoculated after two days and every subsequent day to the fifteenth day inclusive. It is admitted that the withdrawal of the dose for counting by a syringe though graduated in hundredths of a c.cm. is not a very accurate method but it was convenient and suited to the double purpose of this research.

The counting of the plates was done after 48 hours and by a different member of the Commission. The highest figure observed, 363 million per c.cm., was reached on the ninth day, though possibly if counts had been done on the eleventh or twelfth days they might have been higher. The fall in the count between the third and fourth days was also noted in another bottle, whilst in a third bottle it was not noted. Otherwise the tendency is towards a steady rise reaching a plateau about the seventh, eighth or ninth day. In the third bottle above mentioned the increase from the end of the first day to the sixth day is roughly in arithmetical progression.

The guinea pig inoculations showed curious results. In Graph 1 it will be seen that 0.5 c.cm. produces marked local infiltrations at two periods, on the sixth day and on the fourteenth and fifteenth days. The admixture of six units of antitoxin did not prevent the reaction on the fifteenth day. There is no obvious correlation with the number of living organisms. The effects of inoculation from this bottle are summarized here :—

Days of Incubation.	Results of Inoculations of Guinea Pigs.
2	No infiltration. Weight increased from 201 to 237 grams in six days
3	No infiltration. Weight increased from 330 to 378 grams in five days
4	No infiltration but a small lump on second day, disappearing on fifth day. Weight increased from 292 to 304 grams by fourth day
5	Slight infiltration (+) developing into a pea-like lump, which disappeared by the tenth day. Weight increased from 240 to 265 grams by the third day and to 287 by the tenth day.
6	In 24 hours an extensive soft longitudinal infiltration (+++) in abdominal wall, more marked after 48 hours, becoming firmer by the fourth day and extending from pubes to ensiform, developing finally into a discrete subcutaneous lump which did not burst externally but gradually decreased in size though still quite large after three weeks. Weight decreased from 264 to 232 grams by the second day, rising only to 269 grams by the seventh day
7	No infiltration but a very small lump noted on fourth day. Weight increased from 222 to 230 grams on third day, but was down to 206 grams on sixth day, rising again to 254 on tenth day
8	No infiltration, but a small lump noted on fifth day, disappearing promptly. Weight fell from 230 to 217 grams by fifth day, and then rose.
9	No infiltration. Weight increased from 213 to 223 grams by fifth day
10	Slight infiltration (+) followed by a longitudinal band of thickening on third day, which localized to a small lump on seventh day. Weight fell from 207 to 147 grams by third day, and rose to 197 grams by seventh day
11	No infiltration. Weight about stationary for four days then rose
12	No infiltration. Weight fell from 205 to 196 grams in three days
13	No infiltration. Weight fell from 210 to 201 grams in two days
14	Extensive soft infiltration (+++) with oozing of serum from the needle hole, becoming a firmer longitudinal band after three days, central skin necrosis after four days, then an ulcer which had healed by the sixteenth day. Weight decreased from 212 to 181 grams in three days, rising to 257 grams after seven days
15	Moderately extensive infiltration (++) becoming firmer and band like after three days, then settling down to form two lumps, one rather deep below costal margin, the other superficial in the lower abdominal wall. The animal was killed on the sixth day, and these lumps were found to be two abscesses connected by a thin band of tissue. No lesions noted in other organs. Weight fell from 210 to 193 grams in two days
	Another animal was inoculated with 0.5 c.cm. plus 6 units antitoxin. It also developed a definite infiltration (++)

Graph 1 M.S.C. 4. Periodic enumeration of the Bundaberg Staphylococcus growing in T.A.T. at 80° Fah. (26.6° C.) and the results of subcutaneous inoculations of guinea pigs with 0.5 c.cm. (0.Tr. + ++ and +++ indicate increasing degrees of local lesion.)

Looking through the weights of animals inoculated with this and other bottles, the main facts elicited are that inoculations after two, three and four days incubation at Bundaberg temperatures as a rule do not cause loss of weight, inoculations after longer incubations may or may not cause loss of weight, but when the infiltration is severe the loss of weight is usually prompt and definite. Recovery and gain of weight occur after a variable period. The injections from this bottle did not cause death in doses of 0.5 c.cm.

Another rubber-capped bottle inoculated with the Bundaberg staphylococcus gave a somewhat similar sequence of effects. This bottle was kept at room temperature in hot weather, the maximum bench temperature during the eleven days being 93° F. the minimum 65°, whilst the average bench maximum and minimum for the period were 82° and 72° respectively, therefore not far removed from the Bundaberg temperatures (See Appendix 7). The inoculum, counted by plating, amounted to 140 cocci per c.cm. of toxin-antitoxin. The results of subcutaneous inoculations of 0.5 c.cm. are tabulated :—

Days of Incubation.	Results of Inoculations of Guinea Pigs.
2	No infiltration. Weight fell slightly, from 295 to 287 grams in five days, then rose to 313 grams by tenth day
3	No infiltration. Weight increased from 255 to 280 grams in five days, and to 295 grams by ninth day
4	No infiltration. Weight increased from 244 to 287 grams in four days, to 303 grams in six days, then fell to 282 grams by eighth day
5	No infiltration but after three days a small firm lump was noted. It did not ulcerate and gradually grew smaller and was not palpable after seven days. Weight fell slightly from 243 grams to 237 grams in five days, still 237 grams in seven days, and only 247 grams in nine days
6	Extensive infiltration (++++) which persisted for several days, then settled down to a firm subcutaneous lump which disappeared by the ninth day. Weight increased from 242 grams to 286 grams in four days, fell to 265 on sixth day and rose to 301 grams by ninth day. This gain is exceptional
7	Slight infiltration (+) settling down to a longitudinal band of thickening and later to a firm pea-like lump. Weight fell from 287 grams to 266 grams in five days, then rose to 307 grams by eighth day. The animal was killed by ether on the thirteenth day. The lump proved to be a small well enclosed abscess containing whitish rather thick pus. Adrenals normal looking. No other lesions found. A film of the pus showed numerous gram positive cocci and pus cells and much phagocytosis. A staphylococcus was cultivated
8	No infiltration, but a very small lump noted on fourth day. It could not be felt on fifth day. Weight fell from 275 to 255 grams in four days, then rose to 289 grams by the eighth day
9	An extensive soft subcutaneous infiltration (++++) and some redness of skin within 24 hours. On third day it was firmer. On fourth day it was incised and bled freely (hæmorrhagic exudate) but no pus was found. Cultures from the incised tissue gave several colonies only of a staphylococcus. The wound began to heal although the swelling remained. On the tenth day it had resolved into two firm raised lumps The animal was killed by ether and dissection showed a superficial abscess in the mid abdominal wall and a deeper abscess higher up affecting the muscle. The inflammation had caused adhesion of the anterior surface of liver to the abdominal wall and there were several superficial miliary abscesses in the liver. Staphylococci were cultivated from the abscesses, but the heart's blood was sterile. There was no lesion of the adrenals. The weight had fallen from 294 to 243 grams in four days, was only 249 grams after seven days and 262 grams after ten days.
10	Slight infiltration (+) settling down to a small lump in three days. This lump was still palpable after ten days, but had disappeared by sixteenth day. The weight increased from 277 grams to 297 grams in four days, and to 314 grams in six days.
11	Slight infiltration after 24 hours but extensive (++++) on second day. A deep incision caused free bleeding but no pus was found. The wound healed well but the swelling remained and settled down to a raised lump free from surrounding infiltration by the eleventh day. It then showed fluctuation and settled down partly by discharge and partly by absorption. The weight fell markedly from 261 to 177 grams in four days, was only 181 grams on fifth day, and 197 grams on eleventh day
12	This animal was given the remaining contents of the bottle, viz., 1.4 c.cm. In 24 hours its abdomen showed an extensive oedematous swelling which oozed serum from the needle hole. On the second day it was much the same, and on the third day the animal looked very ill. It was killed by ether. Dissection showed oedematous subcutaneous tissue and necrotic muscle. The subjacent anterior surface of liver showed some greyish exudate. The organs were somewhat wet. Adrenals not hæmorrhagic, but slightly congested. Staphylococci were cultivated from the heart's blood and from the necrotic tissues. The weight had fallen from 272 to 248 grams in two days and was 245 grams after three days.

Sections of the tissues involved in the local lesion of this last guinea pig showed oedema and hæmorrhage in the subcutaneous tissue, degeneration of striped muscle, and infiltration of tissues with Gram-positive cocci and leucocytes. Sections of the adrenals showed no abnormality.

In this series the results indicate an initial innocuous period of four or five days duration, next a short period in which there is a severe local effect, then a period of lessened effect and finally a second active period (possibly more permanent) in which severe local effects are obtained. This bottle gave the highest counts of cocci which have been observed in toxin antitoxin of Batch

86. It was the first bottle to be investigated in this manner. Counts of over 2,000 millions per c.cm. were reached though 400 million was the maximum observed in three other bottles. Possibly the organism in our later experiments had lost some of its ability to grow in toxin-antitoxin. Whether this could be regained by passage through successive bottles of toxin-antitoxin mixture has not been investigated.

Another bottle of Batch 86 inoculated with approximately 700 cocci per c.cm. and incubated at 80° F. showed a steady uninterrupted increase in the numbers of living cocci from the second to the sixth day. The results of plating and the effects of subcutaneous inoculations of guinea-pigs with 0·5 c.cm. are here detailed :—

Days of Incubation.	Numbers of cocci in millions (Plate counts).	Results of Inoculations of Guinea Pigs.
1 (21 hours) ..	4½	Not inoculated
2 (51 hours) ..	92	Doubtful slight infiltration next day, but nothing on second day. (Either trace or 0). Weight increased from 269 to 300 grams in three days
3	130	No infiltration. Weight remained about stationary during subsequent nine days
4	154	No infiltration. Weight increased from 220 to 289 grams in three days, then fell to 258 grams by the sixth day and to 254 grams by the eighth day
5	173	No infiltration. Weight increased from 258 to 287 grams in two days then fell to 265 grams by the seventh day
6	231	No infiltration but a very small thickened area was noted after three days and four days, but was gone by the fifth day (tr.). Weight fell from 227 to 215 grams in three days, then rose to original level by fifth day and to 247 grams by seventh day
7	Not counted ..	A definite but slight infiltration (+) on the next day, not increased on second day. On third day it was noted as a longitudinal band of thickening which became firmer, then a broken patch of skin with thickening beneath which was found discharging pus on the seventh day, and was almost healed by the tenth day. The weight fell from 200 to 144 grams in three days, was 167 grams on fifth day and 207 grams on seventh day
8	Not counted ..	Extensive infiltration (++) which was felt from pubis to ensiform, but was less on second and third days. A longitudinal band of thickening was noted on fourth day continuing until seventh day. By the tenth day it had gone on to ulceration and discharge, healing completely later. The weight fell from 212 to 165 grams in two days, was 165 on fourth day, 177 grams on sixth day, and 225 grams on tenth day.

The remaining contents of this bottle were centrifuged. The organisms deposited from 1 c.cm. were emulsified in saline and inoculated subcutaneously. No local lesion resulted. Another guinea-pig was inoculated with the organisms from 0·5 c.cm. emulsified in saline and mixed with 0·5 c.cm. of the super-natant fluid. On the following day there was definite infiltration which persisted for three days and then settled down to a well-defined lump.

In the series above described, no definite infiltration was produced until the seventh, eighth and ninth days, and the local lesions were not as severe as in the two preceding series. The centrifuged organisms emulsified in saline did not produce infiltration.

Another series was carried through with the inoculated bottle in an incubator set at 37° C. The inoculum was approximately 1,000 cocci as determined by plating. There was slight turbidity within eighteen hours. The results were as follows :—

Days of Incubation.	Results of Inoculations of Guinea Pigs.
1 (22 hours) ..	Definite infiltration (between + and + +) next day, not increased on second day, settling down to a small lump which was palpable until the seventh day. The weight decreased from 201 grams to 159 grams in three days, was 183 grams on fifth day and 194 grams on twelfth day.
2	Doubtful slight infiltration (tr.). The weight increased from 190 to 210 grams in two days, to 228 grams in four days, and the 256 grams in six days.
3	No infiltration. The weight fell from 207 to 179 grams next day and rose to 208 grams by third day.
4	No infiltration. The weight was stationary for two days
5	Very slight infiltration 24 hours, but more definite next day (+), and persisted until fourth day, after which it settled down without forming any lump. The weight fell from 204 to 181 grams in three days, then rose
6	No infiltration. The weight increased from 185 to 207 grams in two days, but was only 204 grams on seventh day.
7	No infiltration. The weight fell from 217 to 212 grams next day, but rose to 244 grams by sixth day.
8	No infiltration Weight was 197 on day of inoculation and only 206 grams six days later

The counts obtained in this series at 37° C. were surprisingly low and irregularly variable from day to day, the maximum count being 129 millions per c.cm. Infiltrations were obtained with the toxin-antitoxin after 22 hours incubation and lesser reactions after two and five days, but not after three, four, seven, eight and nine days.

To another bottle was added half the percentage amount of iodine present in the remains of Dr. Thomson's bottle (Appendix 19) (the concentration found was probably a maximum reached gradually during the series of inoculations at Bundaberg). The actual amount of iodine added to the 10 c.cm. bottle was 0·5 c.cm. of a solution containing 0·4 m.gm. per c.cm. The maximal count obtained in this bottle, incubated at 80° F. was 200 millions per c.cm., so that there was no definite evidence of any inhibitory effect on growth with this concentration of iodine. Guinea-pigs were inoculated every day from the third to the fourteenth day, excepting the fifth, eighth and twelfth days. No infiltrations resulted, but the inoculations on the seventh day produced a late and very small ill-defined superficial lump, and that on the tenth day produced a somewhat larger lump, first noted on second day and persisting until on the eighth day it was found discharging a little.

It is unfortunate that inoculations were not done on the sixth and eighth days, and considering this omission we are not justified in assuming that the presence of this amount of iodine inhibits the production of the local lesions in guinea-pigs.

Other investigations were carried out on the growth of the Bundaberg staphylococcus in an antitoxin dilution of the same strength as in toxin-antitoxin mixture, the toxin being replaced by nutrient broth diluted one in five (Appendix 25). Counts were made at intervals and the figures obtained were higher than in most bottles of toxin-antitoxin. The results were as follows:—

Day.	Numbers of cocci (by plating).
2	144 millions
3	227 ,,
4	443 ,,
5	403 ,,
6	636 ,,
7	560 ,,
8	..
9	694 millions
10	482 ,,

A few guinea-pigs were inoculated from this bottle and two others of the same preparation, but unfortunately inoculations were not carried out during the first eight days. No reactions were obtained on the ninth and tenth days; on the eleventh day one bottle gave a definite infiltration (+ +) and the other two did not, and on the twelfth day the former did not, whilst one of the others produced a definite infiltration (+). On the thirteenth day neither of these produced any reaction.

Similarly a dilution of diphtheria toxin was made up of the same strength as in toxin-antitoxin, antitoxin being replaced by 1 in 1,350 horse serum. The same inoculum was introduced as was used above for the antitoxin and a few counts were made. The figures obtained were :—

Day.	Numbers of cocci (by plating).
2	172 millions
3	170 ,,
4	301 ,,
5	242 ,,

Obviously it was not possible to investigate staphylococcal reactions in animals without first destroying the diphtheria toxin, and there seemed to be no useful purpose to be pursued along these lines. The figures are given here for comparison with growth in antitoxin (Appendix 25).

It may be contended that the variations in the reactions of individual guinea-pigs described above are only the expression of variation of susceptibility in the animals themselves. We did not have sufficient animal accommodation at our disposal to enable us to inoculate several animals each day from each bottle of toxin-antitoxin mixture, as we were using many guinea-pigs daily for the titration of diphtheria toxin and antitoxin. We have been able to show, however, that while individual variations in the susceptibility of guinea-pigs do occur they are insufficient to account for our results.

In young guinea-pigs of about 200 grams weight which have been used in all the later experiments, variations have been found in groups of three guinea-pigs inoculated from two bottles of toxin-antitoxin on different days. Increasing degrees of reaction are expressed as 0 (no reaction), tr, +, + + and + + + :—

Group 1	0, 0 and +
Group 2	0, tr. and +
Group 3	Tr, tr. and tr.
Group 4	Tr, tr, and +
Group 5	+, + and +
Group 6	+, + and + +
Group 7	+, + and + +
Group 8	+, + + and + + +

We now attempted to ascertain whether when the staphylococcus was grown in richer media in the absence of toxin and antitoxin the same phenomena could be produced. For this purpose we used tubes containing 10 c.cm. of broth inoculated with 1 loopful of standard emulsion (approximately 1,000 millions per c.cm.). After growth for various times at Bundaberg temperature the broth was well shaken up and most of it filtered through Seitz filters, using discs through which bacteria could not penetrate. The filtrate and the unfiltered material were injected subcutaneously into different guinea-pigs.

In no case between the twelfth hour and the eighth day did filtrate from any of the cultures in doses of 2 c.cm. cause any lesion, though after 30 and 40 hours growth the injection caused some slight loss of weight.

The unfiltered cultures in a dose of 0·5 c.cm. produced the following results :—

Age of Culture.	Local lesion.	Remarks.
12 hours	Nil	No loss of weight
19 hours	Nil	Slight loss of weight
23 hours	Extensive soft infiltration (+ + +) followed by necrosis of skin on third day	Regaining weight by seventh day
28 hours	+ + Infiltration. Ulceration and discharging abscess by third day	Weight regained by sixth day
30 hours	+ + Infiltration	Weight regained by eighth day
40 hours	+ + Infiltration	Weight regained by fourth day
3 days	+ + + Infiltration. Necrosis by second day	Weight regained by fourth day
4 days	+ + Infiltration	Weight regained by sixth day
6 days	+ + + Infiltration	Weight regained by sixth day

As the filtrates were in all cases inocuous even in large doses, we next studied the effect of adding increasing numbers of organisms to filtrates and to sterile broth which were injected subcutaneously. 0·5 c.cm. of standard emulsion, equivalent to 500 million organisms, with 0·5 c.cm. and 2·0 c.cm. of unfiltered sterile broth gave infiltrations in all respects similar to those recorded with unfiltered broth cultures. 0·1 c.cm. of broth plus 0·5 c.cm. of standard emulsion gave only a trace of infiltration and similar but less marked reactions were produced with 0·5 c.cm. standard emulsion and 0·5 c.cm. of filtrate from 24 hours' broth culture. Standard emulsion alone in doses from 0·5 to 1·0 c.cm. never produces any trace of infiltration.

We next attempted to reproduce the phenomenon by emulsifying the organisms off an agar slope eighteen hours old, in 3 c.cm. toxin antitoxin mixture. This emulsion was estimated to contain very approximately 16,000 million staphylococci per c.cm. A series of dilutions were made of this emulsion using toxin antitoxin mixture as a diluent and 0·5 c.cm. of each was injected into pairs of guinea pigs of about 200 gm. weight. The following results were obtained, the degree of infiltration being indicated as heretofore :—

Number of organisms (in millions) injected.	Local lesion after 16 hrs:	40 hrs:	64 hrs:
8,000	+ + + +	+ + +	+ + +
	+ + + +	+ + +	+ +
4,000	+ + +	+ + +	+ + +
	+ + +	+ + +	+ + +
2,000	+ + +	+ +	+ +
	+ + +	+ +	+ +
1,000	+ + +	+ +	+ +
	+ + +	+ + +	+ +
500	+ +	Found dead	
	+ +	+	+
250	tr.	tr.	tr.
	+ +	+ +	+ +
125	+	+ +	+ +
	+	+ +	+ +
62	+	0	0
	0	0	0

While our plate counts in the experiments with toxin-antitoxin mixture inoculated with staphylococci indicate that the number of living organisms is not the explanation of the phenomenon we have described, it may well be that the total number of living and dead organisms is the determining factor in the production of infiltration.

Is Toxin Liberated from Toxin Antitoxin Mixture by the Staphylococcus?—It might be thought that the lesions we have described in guinea pigs were due to diphtheria toxin liberated from toxin-antitoxin mixture by the activity of the staphylococcus. It is quite certain that this is not the case since broth cultures of the staphylococcus, and cultures in antitoxin alone, produce similar lesions. Further, the infiltrations produced by subcutaneous injection cannot be prevented by the simultaneous administration of large doses of antitoxin. Toxin-antitoxin mixture in which staphylococci have been grown has at no time produced intradermal skin reactions in guinea pigs when injected, in doses of 0·2 c.cm. No late paralyses have followed in any of the guinea pigs injected subcutaneously with doses of 0·5 to 1·0 c.cm., and finally the microscopic examination of the infiltrations has shown them to resemble cellulitis of staphylococcal origin. The large series of experiments which we have made with staphylococci growing in toxin-antitoxin mixture under various temperature conditions and the experiments described in Appendix 21 with B. proteus definitely exclude the possibility that toxin can be liberated by the action of proteolytic organisms and in particular by the Bundaberg staphylococcus.

Experiments with Monkeys.—We did some further experiments with monkeys, which we hoped would prove to be more susceptible to the Bundaberg organism than other laboratory animals. Unfortunately this was not the case, but the general results obtained by the subcutaneous injection of toxin-antitoxin mixture injected with the staphylococcus and incubated at 73° to 85° F. for varying periods, were on the whole confirmatory of our observations on guinea pigs. Our first experiments were carried out on the same three monkeys which had been already used for testing the contents of Dr. Thomson's bottle. They had then shown no ill effects. One of these animals on each day received a dose of 1.0 c.cm. from each of two bottles infected with the staphylococcus, in the right thigh from one, and in the left from the other bottle.

Monkey 2, who received his inoculations on the third day of incubation, remained perfectly well, showed no rise of temperature and developed no local lesion at the site of inoculation.

Monkey 1, who received his inoculations on the fourth day, had slight pyrexia on the first and second days after inoculation. His general condition was poor for several days and within 24 hours of the injection there was a firm swelling in the left groin which appeared to consist of swollen glands; by the fourth day a definite abscess appeared to be forming. By the second day a slight firmness was apparent in the adductors and hamstrings in the right thigh. This gradually developed and formed a very large abscess which with that on the left side was opened on the tenth day and a quantity of pus was evacuated.

Monkey 3, who received his inoculations on the fifth day of incubation did not show any pyrexia following his injections but was definitely sick for two or three days. On the third day a painful lump appeared in the right groin. This had cleared up by the sixth day when an area of hard infiltration was observed for the first time in the adductor muscles of the right thigh. By the ninth day this had cleared up and the monkey remained perfectly well.

Further experiments were made on much younger animals which had not had any previous injection.

Monkey 5, weighing 2·1 kilos, received in the right thigh a subcutaneous injection of 0·5 c.cm. of toxin-antitoxin mixture infected with staphylococci and incubated 55 hours, and in the left thigh a similar dose of material, incubated 31 hours. The animal's temperature was slightly elevated (39·6° C.) on the first day, though there were no marked general symptoms. On the second day there was a small hard lump on the left side at the site of the injection, but this disappeared by the fifth day. On the right side there was no definite lesion except some firmness in the adductor region on the second day.

Monkey 6, weighing 2·27 kilos, received in the right thigh a subcutaneous injection of 0·5 c.cm. of toxin-antitoxin infected with the staphylococcus and incubated for three days at room temperature. In the left thigh a similar dose of material incubated for two days was given. There was no definite general reaction, but a firm infiltration of the adductor muscles on the right side appeared on the first day and persisted to the twelfth day. On the left side a small subcutaneous lump developed and persisted to the fifth day.

Monkey 7, weighing 2·35 kilos, received in the right thigh a subcutaneous injection of 0·5 c.cm. of similar infected material incubated four days, and in the left thigh material incubated for three days. On both sides a firm subcutaneous lump appeared associated with some induration of the adductor muscles. These lesions had disappeared by the ninth day.

Monkey 8, weighing 3·11 kilos, received similar doses of material incubated four and five days respectively. No local lesions resulted and there were no general symptoms.

Monkey 9, weighing 1·42 kilos, was inoculated subcutaneously in each thigh with 0·5 c.cm. of toxin-antitoxin mixture infected with the Bundaberg staphylococcus, incubated for four days at room temperature and not disturbed in any way till one hour before it was used. (An attempt was thus made to imitate the conditions in Dr. Thomson's bottle). The general reaction following these injections was very slight and the local lesions were definite but transitory.

Twenty-five minutes after the subcutaneous inoculations approximately 4·0 c.cm. of blood was withdrawn from the heart with a syringe and the blood distributed in graded quantities both into broth tubes and into melted agar, the latter being poured into petri dishes. No staphylococci were cultivated from any of these fractions of the 4·0 c.cm. of blood. In a previous experiment with another monkey no staphylococci were cultivated from approximately 3·0 c.cm. of heart blood 1½ hours after a subcutaneous inoculation.

Twelve days after the inoculation this monkey went off his food and became quiet and listless. On the fourteenth day it weighed only 1 kilo. It had some diarrhoea, refused food and was moribund on the sixteenth day. After death by chloroform an autopsy was performed. There were no abscesses in the thighs or in the internal organs. The heart blood was sterile. The liver showed numerous greyish white flecks, of irregular size, under the cupoula but on incision no pus was found and cultures from them remained sterile. Microscopical examinations of sections showed a heavy coccidial infection. The spleen and kidneys showed no lesion. The mucous membrane of the colon was slightly swollen and of a light pink colour but without macroscopic ulceration. The mesenteric lymph glands were markedly swollen and firm and only colon bacilli were cultivated from them.

These experiments, though carried out upon relatively insusceptible animals, suggest that at temperatures approximating to those at Bundaberg, there is an optimum time for the development of pathogenicity of toxin-antitoxin mixture infected with the staphylococcus.

Generalization of staphylococcal infection cannot be produced by the subcutaneous route in macacus rhesus.

APPENDIX 27.

ON THE DEVELOPMENT OF TURBIDITY IN TOXIN-ANTITOXIN MIXTURE IN RELATION TO THE NUMBER OF STAPHYLOCOCCI PRESENT IN DR. THOMSON'S BOTTLE ON 27TH JANUARY, 1928.

The Bundaberg staphylococcus forms a "smooth" growth both in broth and in toxin-antitoxin. A slight deposit appeared at the bottom of the amber coloured rubber-capped bottles in which the toxin-antitoxin was kept during the experiments recorded in Appendix 26, but the haziness or turbidity in the supernatant liquid was usually free from visible particles. To detect very slight haziness it is necessary to examine the amber bottles side by side with a sterile bottle. Dr. Thomson stated in evidence before us that on 28th of January (the day after the inoculations) he noted that the contents of the bottle were turbid though on the afternoon of the 27th of January (when he made the inoculations) it had been clear. He had in his possession only one bottle of toxin-antitoxin and, therefore, had no standard of comparison. From a considerable experience of turbidity in these bottles and from the very strong probability that the contamination took place not later than the 24th January, we consider that some degree of turbidity was present on the 27th January. The time in which turbidity appears depends on the number of cocci injected through the rubber cap into the bottle, upon the temperature of incubation and possibly upon other factors which we have not elucidated. We endeavoured to obtain a uniform inoculum using a known dilution of a saline emulsion made with a platinum loop from an 18 to 24 hours growth on agar. The number of living organisms was controlled by plating.

At temperatures between 75° and 85° F. (24° to 29° C.) slight turbidity is visible in from 32 to 48 hours with a very small inoculum, e.g., in two experiments in which agar plate counts showed the number inoculated to be 500 and 700 cocci respectively per c.cm. of toxin-antitoxin. At 37° C. the turbidity appears more rapidly, e.g., in one experiment an inoculum of 1,000 cocci per c.cm. determined by plate culture produced slight turbidity within eighteen hours. The turbidity increases but never becomes as great as in nutrient broth, nor did we in an experiment in which the bottle was incubated at 37° C. find any greater turbidity. There is a definite lag in growth under the conditions of these experiments. For example, in an experiment at room temperature (75° to 85° Fah.) with an inoculum of approximately 140 cocci, plate counts after four hours gave 100 cocci per c.cm., after eight hours 450, after 28 hours approximately 3,000,000, and after 46 hours 29,000,000. In another experiment at 80° F. an inoculum of 730 cocci per c.cm. had increased to 4,500,000 in 21 hours.

Even allowing that Dr. Thomson's observations were correct, viz., that the bottle was clear on 27th January, and turbid on 28th January, our experiments indicate that the number of cocci present on 27th January, would have been, on an extremely conservative estimate, not less than 1,000,000 per c.cm. For example, in the experiment with a very small inoculum only 100 cocci per c.cm. were found after four hours, but after 46 hours when very slight turbidity was noted the number of cocci was 29,000,000 per c.cm. *On the day before* turbidity was noted, the count showed 3,000,000. Again, in the experiment with a larger inoculum, enumerated as 730 cocci per c.cm., the count on the day on which turbidity was first noted (but not less than six hours after it appeared) was 92,000,000, and *on the day before* the count showed 4,500,000 per c.cm. Further, in another experiment where the count was made in the afternoon of the day on which turbidity was first noted, the number was found on this day to be 116,000,000 but it was not counted on the day before. These experiments were either at room temperature (82° to 72° F.) or in an incubator set at 80° F.

But if, as we hold, the contamination of the bottle took place not later than the day on which it had been previously used, i.e., not later than 24th January, then from our experiments it would have been slightly turbid on 26th January, the turbidity depending on the factors enumerated, and probably more turbid on 27th January. Our counts after three days, using small but variable inocula, are in the same three experiments respectively 264, 130 and 265 millions per c.cm.

To obtain these small numbers of cocci we took a fresh saline emulsion which from its opacity we judged would work out somewhere in the neighbourhood of 1,000 million per c.cm. 0·1 c.cm. was added to 9·9 c.cm. of saline and well mixed, and of this 0·1 c.cm. was added to a further 9·9 c.cm. of saline. Either 0·1 c.cm. of this dilution was used as the inoculum or 0·1 c.cm. of a further tenfold dilution. If, as in one experiment, 1,400 colonies were obtained by plating 0·1 c.cm. of the latter, then the inoculum per c.cm. of toxin-antitoxin in the 10 c.cm. bottle was 140 cocci, and the strength of the original emulsion approximately 1,400,000,000 per c.cm. Now we do not know how many cocci were admitted to Dr. Thomson's bottle and it would be unjust to presume that it was above the minimum that would succeed in multiplying

sufficiently to cause turbidity. The possibly longer lag in this case and the small initial number of organisms might well have ensured smaller numbers, if counts had been made, than we have obtained. Our smallest estimate after three days at the specified temperatures was 130,000,000 per c.cm. Dividing this by three, as we have done in the consideration of our first hypothesis (on which we presumed Dr. Thomson to have been correct in his observations as to turbidity), we find on this second hypothesis (on which we presume that the bottle was contaminated on 24th January) that the bottle would have contained not less than 40,000,000 per c.cm. on 27th January. This again is an extremely conservative estimate.

If Dr. Thomson had contaminated the bottle only during the final inoculations on 27th January, then with a very small inoculum it would not have become turbid on 28th January, when he noted it, unless the inoculum had been a heavy one, a contingency which we consider extremely unlikely. (See Appendix 3.) Contamination on the 27th., moreover, would not explain the abscess formation in the arms of all the children who survived.

A further observation was made on the turbidity of toxin-antitoxin in amber bottles. An addition of Bundaberg staphylococci to toxin-antitoxin so that it contained not less than 8,000,000 per c.cm. could not be detected by two of us. A concentration of not less than 12,000,000 could just be detected by two of us by careful observation and comparison with a control bottle in a suitable light. A concentration of not less than 20,000,000 could be detected by a third trained observer without previous practice with these bottles. But even a concentration up to 80,000,000 per c.cm. does not cause gross turbidity and might easily be missed if the contents were not closely observed.

APPENDIX 28.

INVESTIGATION OF CONTENT OF DIPHTHERIA ANTITOXIN IN THE SERA OF THE SURVIVORS OF THE INJECTION OF TOXIN-ANTITOXIN MIXTURE ON 27TH JANUARY AT BUNDABERG.

For this purpose two methods were used—(1) the protection of guinea pigs of approximately 250 grams weight against the subcutaneous injection of the certainly lethal dose (C.L.D.) of a sample of diphtheria toxin by its mixture with varying doses of the sera to be tested and, (2) the intradermal method first described by Romer and his colleagues (1909).

The toxin used, pooled 21A and 21D of the Commonwealth Serum Laboratories, was similar to that used in the manufacture of Batch 86. 21A was planted on 12th October, 1926, with the C.S.L. strain of Park 8 and 21D with R.G.W. from the Lister Institute. 21A was collected 20th October, 1926, and 21D on the following day. They were later pooled. This toxin, approximately seventeen months old, was used in all our experiments. It had an L+ dose of ·225 c.cm. when tested at the Commonwealth Serum Laboratories on 13th December, 1927, but when tested by us in March, the L+ dose was found to be approximately ·250 c.cm. We did not accurately determine its M.L.D., but this evidently was not far removed from its C.L.D. The L+ dose for this toxin probably does not contain more than 30 M.L.D's.

For the use of the first method we determined the C.L.D. of the toxin used. This, as will be seen from Table 1, was 0·01 c.cm.

TABLE 1.

DETERMINATION OF C.L.D. FOR TOXIN 21A AND 21D.

No. of Animals Tested.	Dose—Toxin.	Result.
4	·007 c.cm.	Two died on the 7th, 10th days. Two survived.
5	·008 c.cm.	Died on the 3rd, 8th, 13th and 24th days. One survived.
4	·009 c.cm.	Died on the 5th, 5th and 12th days. One survived.
6	·01 c.cm.	Died 40–69 hours.
4	·012 c.cm.	Died 41–72 hours.
4	·014 c.cm.	Died 31–56 hours.
4	·016 c.cm.	Died 28–72 hours.
4	·018 c.cm.	Died 31–46 hours.
4	.020 c.cm.	Died 31–53 hours.
4	·04 c.cm.	Died 31–46 hours.

We next determined the amount of standard antitoxin necessary to ensure survival when injected subcutaneously together with 0·01 c.cm. of toxin. In all cases the mixtures were allowed to stand at room temperature for one hour before injection. Table 2 shows the protective effect of varying doses of standard antitoxin. The standard antitoxin used throughout was prepared by the United States Public Health Service, Hygienic Laboratory, Washington.

TABLE 2.

PROTECTION AGAINST THE C.L.D. (0·01C. CM.) OF DIPHTHERIA TOXIN WITH VARYING DOSES OF STANDARD ANTITOXIN.

Dose of Anti-toxin.	No. of Animals Tested.	Results.
1/200 unit	2	Died on 3rd and 5th days.
1/100 unit	2	Died on 4th and 10th days.
1/70 unit	4	Two died on 3rd and 4th days. Two showed infiltration and necrosis—regaining weight by 14th day. Definite weakness of hind limbs on 21st day.
1/60 unit	4	All showed infiltration and necrosis—regaining weight by 10th day.
1/50 unit	6	All showed infiltration and necrosis. Two lost weight, but commenced to regain it on 7th day.
1/40 unit	3	All showed infiltration, necrosis, and two loss of weight—regained by 13th day.
1/30 unit	4	All showed infiltration and slight necrosis—weight regained by the 6th, 10th and 13th days.
1/25 unit	2	No infiltration—steady gain of weight.

The dose of antitoxin necessary to ensure survival was 1/60 unit antitoxin, while that which gave complete protection (freedom from infiltration and loss of weight) was 1/25 unit.

We were now able from the protective effect of varying doses of the sera to deduce the amount of antitoxin present in them.

For the intradermal method we used the technique described by Glenny and Allen (1921), taking as a standard the reaction produced by the injection of that amount of toxin (Ln/500) which together with 1/500 unit of standard antitoxin just suffices to produce necrosis. The sera were put up each in a series of dilutions mixed with this dose of toxin and the total volume injected was in each case 0·2 c.cm. Mixtures were allowed to stand one hour before injection. Albino guinea pigs were used. The flanks were shaved and four intradermal injections were made on each side, without anaesthesia. In every animal a standardizing injection of the Ln/500 of toxin mixed with 1/500 standard antitoxin was made, and proved of value as there was some individual variation in the sensitiveness of different animals.

Determination of the Ln/500 Dose.—Several experiments were made, the range of doses used being as follows:—·0004, ·0005, ·0006, ·0007, ·0008, ·0009, ·001, ·0012, ·0014, ·0016, ·0018, and ·002 c.cm. of toxin. The smallest dose which with ·002 c.cm. standard antitoxin produced definite necrosis in different animals was either ·0008 or ·0009 c.cm. We, therefore, selected the latter as the Ln/500 dose.

As a test of our ability to obtain satisfactory results by these methods, we examined the sera of a number of children in Melbourne who had previously been Schick tested, and some who had later been partially immunized and retested. The results obtained are shown in Table 3, and the details of the protection tests are shown in Table 4.

TABLE 3.

Result of Titrations of Antitoxin in Sera of Children who had been Schick Tested.

—	—	Age.	Intradermal Tests.	Protection Experiments.
			Schick Negatives.	
1	R.G. ..	3	1 c.cm. contains about 2/5 unit AT. ..	1 c.cm. contains about 1/3 unit AT.
2	H.T. ..	3½	1 c.cm. contains less than 1/40 unit AT.	1 c.cm. contains about 1/42 unit AT.
3	L.A. ..	6	1 c.cm. contains between 1/10 and 1/40 unit AT.	1 c.cm. contains between 1/12 and 1/24 unit AT.
4	G.B. ..	7	1 c.cm. contains about 2/5 unit AT. ..	1 c.cm. contains about 1/3 unit AT.
5	L.A. ..	7	1 c.cm. contains about 2/5 unit AT. ..	1 c.cm. contains less than 1/3 unit AT.
6	D.R. ..	8	1 c.cm. contains about 2/5 unit AT. ..	1 c.cm. contains about 1/3 unit AT.
			Schick Positive.	
7	E.R. ..	2½	1 c.cm. contains less than1 /40 unit AT.	1 c.cm. contains less than 1/60 unit AT.
8	J.C. ..	3	1 c.cm. contains less than 1/40 unit AT.	1 c.cm. contains less than 1/42 unit AT.
9	V.M. ..	4	1 c.cm. contains less than 1/40 unit AT.	1 c.cm. contains less than 1/60 unit AT.
			Previously Schick Positive but Immunized nearly Three Months before Sera Tested.	
10	B.H. ..	4	Now Schick positive. 1 c.cm. contains less than 1/40 unit AT.	1 c.cm. contains less than 1/42 unit AT.
11	G.K. ..	4	Now Schick negative. 1 c.cm. contains more than 2/5 unit AT.	1 c.cm. contains between 1 and 2 units AT.
12	G.L. ..	4½	Now slight positive Schick reaction. 1 c.cm. contains less than 1/40 unit. AT.	1 c.cm. contains less than 1/42 unit AT.

10, 11 and 12 received immunizing injections of 2, 2 and 4 minims of toxin-antitoxin on 10th November, 25th November and 1st December 1927. The sera were obtained on 27th February, 1928.

TABLE 4.

Protection Experiments with Sera from Schick Positive and Schick Negative Children.

No. of Serum.	Dose of Serum mixed with .01 c.cm. Toxin for injection into guinea-pigs of 250 gm.	Result.
No. 1	0·4 c.cm.	No infiltration—steady increase of weight.
	0·2 c.cm.	No infiltration—steady increase of weight.
	0·1 c.cm.	No infiltration—no loss of weight.
	0·05 c.cm.	Infiltration, necrosis, body weight not regained by 15th day.
	0·04 c.cm.	Died 10th day.
	0·02 c.cm.	Died 7th day.
No. 2	0·7 c.cm.	Marked infiltration—weight not regained by 18th day.
No. 3	0·5 c.cm.	Definite infiltration. No loss of weight.
	0·4 c.cm.	Definite infiltration, no loss of weight.
	0·2 c.cm.	Died 5th day.
No. 4	0·4 c.cm.	No infiltration—no loss of weight.
	0·2 c.cm.	No infiltration—weight regained by 5th day.
	0·1 c.cm.	Slight infiltration—weight regained by 5th day.
	0·05 c.cm.	Infiltration—weight regained by 11th day.
	0·04 c.cm.	Died 5th day.
	0·02 c.cm.	Died 5th day.

TABLE 6.

DETAIL OF PROTECTION EXPERIMENTS WITH SERA OF CHILDREN WHO SURVIVED INJECTIONS ON 27TH JANUARY, 1928.

Initials and Case Number.	Dose of Serum mixed with .01 c.cm. Toxin for Injection into Guinea Pigs of 250 G.	Result.
B.D. (Case 17) ..	0·7 c.cm.	Died in less than 63 hours.
E.D. (Case 21) ..	0·7 c.cm.	Died between 72 and 87 hours.
J.S. (Case 7)	0·2 c.cm.	Marked infiltration, necrosis. Regained weight by 14th day.
	0·15 c.cm.	Marked infiltration, necrosis. Regained weight by 14th day.
	0·1 c.cm.	Definite infiltration. Not back to original body weight by 14th day.
W.S. (Case 6) ..	0·3 c.cm	Only slight infiltration. No loss of weight. Survived.
	0·2 c.cm.	Definite infiltration. Regained weight by 8th day.
	0·1 c.cm.	Died between 72 and 87 hours.
B.P. (Case 13) ..	0·4 c.cm.	Slight infiltration, steady gain in weight.
	0·35 c.cm. (2 animals) ..	Infiltration. No definite loss of weight.
	0·2 c cm.	Infiltration, necrosis. Loss of weight. Died 8th day.
	0·1 c.cm.	Died 9th day.
V.T.C. (Case 10) ..	0·7 c.cm.	Died between 72 and 87 hours.
F.B. (Case 2)	0·03 c.cm.	No infiltration, steady gain of weight.
	0·02 c.cm.	Infiltration, necrosis. Regained weight by 14th day. Survived.
	0·01 c.cm.	Infiltration, necrosis. Loss of weight Died 14th day.
	0·006 c.cm.	Died 3rd day.
	0·004 c.cm.	Died 4th day.
N.W. (Case 9) ..	·006 c.cm.	Infiltration. No loss of weight.
	·005 c.cm.	Infiltration, necrosis. Regained weight by 8th day. Survived.
	·004 c. m.	Infiltration, necrosis. Regaining weight 15th day. Survived
	·003 c.cm.	Died 4th day.
	·0025 c.cm.	Infiltration. Steady loss of weight. Moribund 18th day.
	·002 c.cm.	Died 8th day.

It will be noted that in two of the three sera of children in the first group who had no symptoms there was a particularly high content of antitoxin. The fourth child, C.W., who had no symptoms and from whom we failed to obtain blood had had diphtheria thirteen months before. Her sister, N.W., who had the highest observed titre of antitoxin was also said by her parents to have had diphtheria. These high titres of antitoxin were observed too soon after the last injection to have resulted from active immunization in Schick positive individuals. The children probably had a high titre of antitoxin before 27th January, the injection acting as a secondary stimulus. It may be argued that protection against diphtheria toxin was the cause of their freedom from symptoms. The absence of symptoms in V.T.C. cannot be explained in this way.

With regard to the children in the second and third groups, it is not possible to infer from the antitoxin content of their sera on 15th February whether they were Schick positive or Schick negative before 27th January. In the second group the effect of the intramuscular injections of 4,000 units of antitoxin given while in hospital is still evident in the results of these titrations, and it is possible that had they not had antitoxin the content of their sera in antitoxin would have been similar to that of those in the third group.

On the whole, these results are unfavorable to the view that free diphtheria toxin played any active rôle in causing the Bundaberg deaths.

APPENDIX 29.

THE EFFECT OF THE SUBCUTANEOUS INJECTION OF DIPHTHERIA TOXIN TOGETHER WITH THE BUNDABERG STAPHYLOCOCCUS.

Although we have been unable to dissociate toxin from toxin-antitoxin mixture by growing organisms in it, we thought it advisable to make a few experiments to ascertain whether the resistance of animals could be so reduced by diphtheria toxin that rapid death would result from the injection of the Bundaberg organism. We have used only subcutaneous injections of both toxin and staphylococci in mice, guinea pigs and rabbits, injecting both in the same region. The staphylococci have been injected in saline emulsion made from eighteen to 24 hours old agar slope cultures and estimated by opacity.

In mice we did not determine the C.L.D. of the toxin, but injected in a dose of 0.1 c.cm. it failed to cause any symptoms. Injection of 100 million staphylococci was also without effect both alone and together with 0.1 c.cm. of diphtheria toxin.

In guinea pigs the C.L.D. of the toxin was 0.01 c.cm. (Appendix 28) for animals of 250 grams weight. The injection of 1,000 million staphylococci produces only a local lesion (Appendix 23). In four animals 1,000 million organisms were injected together with 0.002 c.cm. of diphtheria toxin and all survived. Of four animals injected with the same dose of staphylococci and 0.005 c.cm. of toxin, one died on the fourth day and the remainder and the control guinea pigs, which received only 0.005 c.cm. of toxin, survived.

The injection of 200 million staphylococci does not appear to shorten appreciably the time of death after administration of the C.L.D. of toxin. The average death time in ten animals which received 0.01 c.cm. of toxin was 55 hours, and in four guinea pigs which received in addition this dose of staphylococci the average was 52 hours.

In rabbits of about 1 kilo, the C.L.D. was not accurately determined but was probably between 0.005 c.cm. and 0.01 c.cm. 0.1 c.cm. killed in 27 hours, 0.05 in 41 hours, 0.025 in 50 hours and 0.01 in 86 hours, while with 0.005 c.cm. two of three animals died on the fourth and seventh days. Rabbits injected with 0.0025 c.cm. of toxin with and without 1,000 million staphylococci survived, and two rabbits which received 0.005 c.cm. of toxin and the same dose of staphylococci survived beyond the tenth day.

These experiments show clearly that even large doses of diphtheria toxin do not enable the staphylococcus to kill rapidly, when administered by the subcutaneous route in the doses which we have used. It cannot be inferred with certainty from these negative results that an effect of this kind cannot be produced in man, but our evidence makes it unlikely, and experience of staphylococcal infection complicating clinical diphtheria is also unfavorable to this possibility.

APPENDIX 30.

ON GENERALIZED STAPHYLOCOCCAL INFECTION.

By the kind permission of Dr. Cecil Purser, Chairman of the Board of Directors, Dr. A. H. Tebbutt has examined for us the records of the Royal Prince Alfred Hospital, Sydney, in order to obtain some insight into the incidence of the mortality and clinical features of generalized staphylococcal infections. The group studied is classified under the heading "Septicaemia and Pyaemia (non-puerpural)." From the year 1910 to 1928 80 cases have been so classified, but one was definitely a case of sapraemia, and another a readmission, leaving 78 cases; 54 of these have been recorded as septicaemia, and 24 as septico-pyaemia. It must be remembered that the distinction is not always clear, and in many cases the diagnosis has been made on clinical grounds only. It should be emphasized that many conditions other than puerperal (e.g., sub-acute bacterial endocarditis) in which cultures of pathogenic organisms can be obtained from the blood during life, are not included in this group. In only one of the cases studied were there vegetations on the valves of the heart.

Of the 54 cases of septicaemia, 45 died and nine recovered giving a mortality rate of 83·3 per cent. Of the 24 cases of septico-pyaemia fourteen died and ten recovered, giving a mortality of 58·3 per cent. The mortality for the whole series was 75·6 per cent.

In 27 cases cultures were made from the blood during life. In eleven staphylococcus aureus, in six streptococci, in one a pneumococcus, in one a colon bacillus and in eight cases no organisms were cultivated. In two cases the pathologist recovered staphylococci (aureus and albus respectively) which were regarded by him as contaminations. These have not been included as positive cases. Cultures obtained during life from various lesions yielded confirmatory evidence in many cases.

Autopsies were carried out only on 39 cases. Where cultures were made they have supported the diagnosis by blood culture during life, and the same organisms have been obtained from the heart's blood, the splenic pulp, septic infarcts in the lung or miliary abscesses in the kidney.

In a few cases in which blood cultures were not made during life, cultures obtained at autopsy were available for the diagnosis of the infecting organisms. These are as follows:—

Streptococcus, three cases,
Staphylococcus aureus, six cases,
Staphylococcus albus, one case,
Pneumococcus, three cases,
Colon bacillus, two cases.

Adding these to those in which the organism was obtained by blood culture, we find that the infecting organism has been determined in 34 cases as follows:—

Staphylococcus aureus,	17 cases	+52·94 per cent.
Staphylococcus albus	1 case	
Streptococcus	9 cases	26·47 per cent.
Pneumococcus	4 cases	11·76 per cent.
Colon bacillus	3 cases	8·83 per cent.

These figures indicate the frequency of occurrence of staphylococci in this somewhat restricted group of general infections.

The mortality rate in the eleven cases in which staphylococcus aureus was obtained by blood culture during life was 100 per cent., whilst that in the remaining eight cases in which other organisms were recovered was 50 per cent. and in the eight cases in which the blood culture proved sterile 62·5 per cent.

A careful study of the cases in this series indicates the gravity of generalized infection by staphylococcus aureus and engenders a very marked respect for it as an infective agent. It is regrettable that from the point of view of accurate diagnosis and prognosis that blood cultures are not more frequently and promptly carried out in severe infective conditions.

The principal features of this series of eighteen generalized staphyloccocal infections are briefly considered :—

1. Age .. .. Seven out of eighteen patients were under the age of twenty The cases are distributed in decades as follows :—

1st Decade—2, aged 14 months and 3 years.
2nd Decade—5, aged 13, 14, 14, 14 and 17 years.
3rd Decade—2, aged 20 and 25 years.
4th Decade—5, aged 33, 33, 37, 37 and 39 years.
5th Decade—3, aged 42, 47 and 48 years.
6th Decade—1, aged 55 years.

2. Sex .. .. There were fifteen males and three females. Apart from this small group, in the whole series the great majority are males. This predominance may be correlated with the greater incidence of septic wounds (trauma) and boils and carbuncles in the male sex.

3. Primary foci .. These were as follows :—

Lesion.	*Number of cases.*
Boils or pustules	5
Mumps and boils	1
Septic wounds, generally superficial and slight	4
Superficial abscesses	2
Deep abscesses (iliac)	1
Cellulitis of the scalp	1
Blepharitis	1
Septic throat	1
No primary focus found	2
	18

4. *Onset.*—The symptoms as a rule are related to a primary septic focus which had sometimes been neglected. The illnesses begin in various ways, sometimes with headache or pains in the chest, abdomen or limbs, occasionally with vomiting or biliousness, feverishness, flushing of the skin and thirst or profuse perspiration. There was a definite chill in only one case and rigors in none. The local lesion sometimes flared up but in some cases only a serous exudate was found in the inflamed tissues. Enlargement of lymph glands is sometimes mentioned. Osteomyelitis or periostitis is noted in a few cases.

5. *Temperature.*—This is usually high but remitting one, two or three degrees daily. Temperatures of 104° F. and 105° F. are reached in most cases, and in many there is a marked rise and in a smaller number a fall before death.

6. *The Pulse.*—The pulse is usually very rapid and soft. In most cases it varies daily from 120 to 160, but usually reaches 180 towards the end, even in adults.

7. *Respiration.*—This is always moderately rapid, varying daily from 25 to 40 on the average but in several cases the rate is much higher, from 40 to 60. It tends to increase towards the end. The rate is apparently influenced by the extent of the pulmonary lesions, and fibrinous or septic pleurisy and plumonary infarcts or abscesses are common. Respiration may be either rapid and quiet, grunting or gasping. Dyspnœa is occasionally a feature.

8. *The Blood.*—The illnesses were often of short duration or the patients were admitted in a very grave condition so that blood cultures were usually done rather late in the disease. In twelve cases the diagnosis was made by blood culture, In no case was the finding of a sterile blood followed by the autopsy finding of staphylococcal septicæmia or septico-pyæmia. The number of days between positive blood culture and death were as follows :—

The day of death	3 cases
One day before death	3 cases
Two days before death	3 cases
Three days before death	1 case
Fourteen days before death	1 case
Fifteen days before death	1 case

Leucocyte counts were done in nine cases and showed considerable variations. In two of the cases there were only 8,000 leucocytes. Most commonly the number was between 10,000 and 20,000 (five cases). In the remaining two cases there were respectively 22,000 and 58,000. In nearly all the cases the count was made within a day or two of death.

9. *The Skin and Connective Tissues.*—In many cases no observations are recorded. In several there is profuse sweating which is sometimes an early symptom. The skin may be flushed, hot and dry. Apparently until near the end the colour may be good though pallor may be present. There may be marked lividity post-mortem. In one case, a boy of fifteen years, two days before death the skin became ashy grey with blue lips, ears and nose and glazed eyes. In another case cyanosis was marked. A notable feature in a number of cases is the occurrence of small and large bullæ on the same limb as the primary focus, or fleeting red patches with œdema particularly on the forearms even though the primary focus be elsewhere. These are apparently metastatic lesions. In some there are secondary subcutaneous abscesses. Secondary infection of joints is distinctly rare as compared with streptococcal and pneumococcal infections. Sometimes there is direct spread of a furuncle into adjacent subcutaneous and even muscular tissues.

10. *Digestive System.*—Troublesome symptoms are uncommon. The mouth is sometimes very dry, the tongue usually furred, the breath sometimes offensive Vomiting if present is usually an early symptom. Diarrhœa is rare. The abdomen is often distended.

11. *Excretory System.*—Albuminuria is noted in several cases, glycosuria in only one case. Oedema of the limbs is always either inflammatory or follows venous thrombosis.

12. *Nervous System.*—Nervous symptoms are often prominent. Delirium is specially noted in seven cases. It is either very early or does not occur until the last two or three days. Not infrequently it gives place to coma towards the end. In another case there were headache and drowsiness deepening to coma. In two others also coma developed apparently without preceding delirium. In another case there was mental confusion. Marked restlessness sometimes accompanies the delirium. A young man of 25 years, on the day of his death "stared wildly with almost fixed pupils, clutching fiercely at objects or bystanders, then relapsed into general convulsive movements or twitchings." Pain is not infrequently noted early in the illness. Stiffness and pain about the local lesion, or pains in the limbs or joints are not infrequent. Pains in the chest are not uncommon and appear to be due to pleurisy or pleuro-pericarditis. Incontinence of urine and faeces are often coincident with states of delirium and coma. The outstanding features are the effects upon the higher centres—stupor, delirium or unconsciousness. This is in strong contrast with the lucid mental condition often seen in streptococcal septicaemia.

13. *Special Features.*—A pleural friction rub or an effusion from which staphylococci were cultivated is not uncommon. Lobar or broncho-pneumonia has been suspected where congestion, infarction or abscess formation have been found at autopsy. Typhoid fever has been suspected but the Widal reaction has been absent when sought for.

14. *Duration.*—This is not always easy to determine. There is evidence that a decided rise in temperature and pulse rate and varying effects upon other systems mark the transition from local infection to septicaemia or septico-pyaemia. There is great variation in the length of the illnesses. One case had a raised temperature for only two days before death occurred. In other cases the duration was apparently 3 days, 6 days (3), 7, 10, 11 and about 21 days (3). Several patients died within 24 hours of admission to hospital but had been ill for a few days in their homes. The patient whose acute illness lasted only two days had nevertheless septic infarcts in the lungs, and a few small abscesses in the kidneys.

15. *Lesions at Autopsy.*—There is no definite evidence that any patient with staphylococcal septicaemia or septico-pyaemia recovered. All the patients in whom the diagnosis was established by blood culture (12 cases) died and nine

of them came to autopsy. These and the remaining six cases in which the diagnosis was made at autopsy make a total of fifteen autopsies. There is no significant difference between the findings in those cases which gave a positive blood culture and those in which blood cultures were not done.

The Heart frequently shows some dilatation particularly of the right side and not infrequently there is greyish or white clot in the right auricle and ventricle. In only one case is ulcerative endocarditis noted and that on the tricuspid valve with ulceration spreading through the adjacent heart wall and finally rupture and haemopericardium. In one other case there was some mitral stenosis, but it was not thought that there was any acute endocarditis. The infarction of the lungs, therefore, is not due to malignant valvular disease. In only two cases is pericarditis recorded.

Lungs.—In most cases there are obvious pulmonary lesions. Fibrinous or septic pleurisy is frequently mentioned. Septic infarction is the most common lesion of the lung itself. The infarcts often stand out prominently and apparently progress rather rapidly to abscess formation. Large branches of the pulmonary artery supplying an extensive infarcted portion of one lobe were found definitely thrombosed in one case. In other cases there is congestion of the lung or collapse associated with a large pleural exudate. Subpleural and interstitial hæmorrhages may also occur. Septic mediastinitis is noted in one case.

Spleen.—Almost invariably the spleen is moderately enlarged and very soft, even diffluent. Sometimes it is noted as enlarged and friable. Hæmorrhages are also described but in no case infarcts or abscesses formation.

Liver.—This organ is usually described as enlarged and congested, sometimes also as fatty. Abscesses are not noted in any case.

Kidneys.—These are generally described as swollen or congested. Multiple abscesses are common, though in several cases there were only a few abscesses. They are generally described as small or miliary. Infarction is described in two cases and hæmorrhages in two.

Alimentary Tract.—Lesions of note are rarely found, though the examination is often incomplete. In one case hæmorrhages into the wall of the small and large bowel are described.

Brain.—In several cases congestion of the external blood vessels is noted, and in one case numerous sub-arachnoid hæmorrhages. In a number of cases it was not examined.

Skin.—In two cases there is special mention of marked lividity of the face and chest In one it is noted that lividity was not confined to the dependent parts. In another there were abscesses and petechiae in the skin of the back and in another blebs in the skin of the forearm.

The Primary Focus.—In two cases dissection of the veins from the lesion showed definite thrombosis.

Post-mortem Cultures.—The heart blood and spleen give possible findings, though in by no means all the cases were cultures made, since the blood had given staphylococci during life. Abscesses of the lung also gave staphylococci. Cultures from the brain were made in one case and gave positive results.

Summarizing these findings one may classify the autopsy findings and divide them into—

(1) Septicaemia in the strict sense, without secondary foci : three cases.
(2) Septico-pyaemia, with infarctions, abscess formations, septic inflammations of serous membranes : twelve cases.

Crypto-genic cases.—

These are rare and constitute an interesting type. There are two in this series.

One patient, a boy of fifteen years, had been feeling " off colour " for a few days, then complained of fleeting pains in the joints, and later on of pains in the chest especially during deep inspiration. There was some dyspnoea. No primary focus was found. On admission to hospital his temperature was 104° F., respiration 35–40, pulse 130. His skin was ashy grey, and his lips, ears and nose bluish. A pleural friction rub was heard over the left base and crepitations over

the right base. He became delirious, showed albuminuria and gave a positive blood culture. Diagnosis was not much helped by the leucocyte count in this case. Three days before death it was 10,000 with 79% neutrophils and 4% eosinophils. It cannot be too greatly stressed that leucocyte counts should be often repeated to bring out their real value in diagnosis and prognosis. Temperature pulse and respiration rate rose steadily, reaching respectively 106·8° F., 190 and 80 on the day of death, five days after admission. There was no autopsy.

The second patient was a man of 33 years, who had a bilious attack with vomiting. On the following day he felt better. On the third day he felt sick again and had abdominal pains without diarrhœa. There were headache, anorexia, feverishness and some cough. On admission the temperature was 102° F., pulse rate 110 and respiration rate 40. The provisional diagnosis was typhoid fever but the Widal test proved negative. The spleen was thought to be enlarged. There was a previous history of rheumatic fever, nineteen years before, and a systolic murmur was detected. Nothing else was elicited in the physical examination. His temperature remained high—103° F. to 105° F.—the pulse only 110, but rising to 180 before death which occurred five days after admission. On the third day staphylococci were cultivated from the blood. The urine contained much albumin. At autopsy the skin showed much lividity. The bases of both lungs were greatly congested. The mitral valve showed some stenosis and there were a few vegetations, which were noted as " not recent," on the fimbriae of the valve cusps. The left ventricle showed hypertrophy and dilatation. The spleen was much enlarged, friable and congested. The liver was large and fatty. Both kidneys showed cloudy swelling, petechial hæmorrhages and one showed two septic infarcts. There were hæmorrhages into the bowel wall, more notably in the colon. These hæmorrhages were " not infiltrated ". Culture from the spleen and heart blood gave staphylococcus aureus. No primary focus was found.

APPENDIX 31.

DEFINITION OF SOME TECHNICAL TERMS USED.

1. ALLERGY is a natural inherited condition of hypersensitiveness to the injection of various substances which is not dependent in any way on antibody production.

2. ANAPHYLAXIS is a special form of hypersensitiveness induced by the injection of substances with antigenic properties, the toxic phenoma following the re-injection of the antigen after a suitable interval of time.

3. ANTIGEN. A substance which on injection into live animals stimulates the production of antibody.

4. ANTITOXIN UNIT (Ehrlich) was originally defined as the smallest quantity of antitoxin that would entirely prevent all the toxic effects of 100 minimum lethal doses of the particular diphtheria toxin which Ehrlich was using at the time.

5. BACTERIOPHAGE (phage). An agent which causes dissolution of the micro-organisms with which it is propagated.

6. C.L.D. (certainly lethal dose) is for any species the smallest dose of a toxic substance which invariably kills an animal of that species.

7. DANYSZ PHENOMENON. A dose of toxin which is exactly neutralized when mixed in one operation with a given quantity of antitoxin, if added to the same amount of antitoxin in successive moieties, results in a toxic mixture.

8. L + DOSE is the least quantity of diphtheria toxin which when mixed with one unit of standard antitoxin and injected subcutaneously into a guinea pig of 250 grams kills within four to five days.

9. M.L.D. (minimum lethal dose) of diphtheria toxin is the least quantity of any given toxin which when injected subcutaneously into a guinea pig of 250 grams kills it in about four days.

10. MÜLLER PHENOMENON (Haemophagie). The appearance on blood agar of a zone of punctate haemolysis round a staphylococcal colony at a small interval from its edge.

11. SCHICK TEST. The intradermal injection of a minute dose of diphtheria toxin to determine by the occurrence of reaction the susceptibility of an individual to diphtheria. A positive reaction indicates that an amount of circulating antibody insufficient to protect the individual is present.

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