

## The Brown Animal Sanatory Institution

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### CHAPTER 5: FINANCE, INCOME AND EXPENDITURE

In a factual report of this kind something must be said about the financial resources of the Institution, if only to show how handicapped a research laboratory is whose income is below that required for its proper functioning; and at the same time how much can be done by men of vision and determination in the most difficult and disheartening circumstances.

As related in Chapter 2 the sum received in 1858 by the University of London after litigation on Thomas Brown's will had been completed was £22 600 invested in 3% Consolidated Stock. By the time the Institution was finally established in 1871 this sum had increased to over £33 000. During the next year or two £3750 was spent on new buildings, repairs and fittings. To increase the income the Chairman of the Management Committee recommended the transfer of the capital to some more remunerative security. This was done; and between 1874 and 1878 the holdings in 3% Consolidated Stock were transferred to 3½% Metropolitan Stock. Expenses of the building programme between 1878 and 1880 demanded the sale of further stock, leaving a capital sum of £26 800.

The annual income of approximately £950 was augmented to some extent by fees charged for the housing of animals in the hospital, by small gifts and legacies from animal lovers, by larger bequests, by grants from various bodies, and by occasional loans from the University. Thus, a legacy of £1300 was specified in the will of Mrs Isabella Papacalo in 1899. Since the value of her estate had been overestimated, the Brown Institution received in 1901 only £516, but after her husband died in 1903 a further sum of £661 was paid over. In 1903 the executors of Mr Reymes Hurrell presented the Institution with a gift of £500. A public appeal for money in 1889 brought in the paltry sum of £294, and a private appeal in 1911 one of only £73. Between 1911 and 1914 the University made available a total of £525 from the Dixon Bequest. In 1921 a sum of £3800 nominal value was allocated to the Institution under the will of Miss F. J. Wedgwood, and a further sum of £286 by the Trustees of her bequest in 1948. In 1910 the Senate recommended a grant to be made from University funds of £300, and in 1912 a special allocation of £284 to help to meet the current deficit. In this latter year the Senate made the Institution a loan of £800 with interest at 3½% per annum, repayment to be made by 30 annual instalments each of £26. 13s. 4d. In 1908 Mrs Lilian Emily Cooke left a legacy of £190, and in 1909 Mrs A. K. Kluth one of £32.

Help was sought in 1910 from the Development Commission. A memorandum was submitted describing the investigations carried out at the Institution bearing

on agriculture and rural industries. Work had been done for the Local Government Board, the Royal Agricultural Society, the Agricultural Department of the Privy Council, the Veterinary Department of the Army, the Hydrophobia Commission, the London County Council, and the Royal Society; and as a result of the Muzzling Order arising from Victor Horsley's work, rabies had been completely exterminated from the country. The Development Commission, however, replied that the nature of this work did not fall within the category of purposes for which they were empowered to recommend advances from the Development Fund.

Grants were received from time to time from the Board of Agriculture, the Royal Agricultural Society, the Association for the Advancement of Medicine by Research, the Grocers' Company, the Royal Society and the Local Government Board, as well as from a few private persons. Most important of all was the personal grant of £600 a year made by the Medical Research Council to Dr Twort from 1920 to 1936.

On the other hand, apart from expenditure on buildings and apparatus, compensation had to be paid in 1911 to the father of a child who was frightened, fell, and cut its face when a chimpanzee from the Institution jumped over the wall, and to the landlady who had a nervous attack. The case was settled out of court for a sum of £132.

Again the sum of £1 a week was paid to the caretaker's wife who had suffered 'terrible disfigurement' as the result of an accident several years previously. After her husband died it was decided in 1921 to pay her £100 in full settlement and discharge of any claims she might make against the University. This undertaking was contingent on her vacating the caretaker's cottage. In 1908 a sum of £119 in cash was found to be unaccounted for. Apparently it had been used by the late Veterinary Assistant for his own purposes. Nothing could be done about it, and the Senate wrote it off as a bad debt.

In 1913, as the result of the failure of an application by the Committee to the Board of Agriculture for a grant of £500 to investigate Johne's disease in cattle, the Vice-Chancellor addressed a letter to the President of the Board asking him if he would receive a deputation from the University. The Board agreed, and on 1 July a strong deputation was sent. It was headed by the Vice-Chancellor (Sir Wilmot Herringham), and the Principal (Sir Henry Miers), together with the Chairman of the Committee of the Brown Institution (Sir Alfred Pearce Gould), the President of the Royal College of Surgeons (Professor Sir Rickman Godlee), Professor Sir John Rose Bradford, Professor Leonard Hill, and Professor S. G. Shattock. In the absence of the President of the Board (Mr Runciman), the deputation was received by the Permanent Secretary (Sir Sydney Olivier) and two Assistant Secretaries. In reply to the case put by the deputation, the Permanent Secretary explained that the Board were already supporting the Royal Veterinary College as well as its own laboratory, and that it was difficult to apply to the Development Fund for the endowment of other institutions. This application for financial support, therefore, proved as fruitless as that to the Development Commission three years earlier.

*State of the finances*

It would serve no useful purpose to follow the state of the finances year by year, unless it was possible to give the reasons for the various fluctuations that occurred. Without far more information than is available in the surviving records this would be impossible.

Broadly speaking, for the first 30 years of its existence the Institution was run on what is popularly expressed as a shoestring. There was an almost continuous deficit. At the turn of the century the state improved, and for about ten years a credit balance was recorded. But from 1911 till 1916 there was again a deficit. During the latter part of the First World War, however, owing to a great decrease in expenditure, a credit balance was again restored. From then onwards, almost without exception, there was a yearly credit balance reaching £1000 in 1945, by which time the Institution had suffered so badly from war damage that it had to be finally abandoned. During the following years the capital accumulated at compound interest till by 1966 it was over £86000. The final disposal of this sum will be described in Chapter 13.

## CHAPTER 6: THE ANIMAL DISPENSARY AND HOSPITAL

The work carried on at the Brown Institution fell into two parts – the routine treatment of sick animals brought to the dispensary, and the research investigations on experimental animals in the laboratory. It is doubtful whether any research was conducted on the sick animals, beyond, that is to say, comparing different methods of treatment; but some of the successive veterinary assistants engaged in experimental work on the laboratory animals, either alone or in collaboration with one of the medical members of the staff. Mr William Duguid, for example, who was a pupil of Professor John Gamgee at Aberdeen, and came with him to London in 1865, co-operated with the Veterinary Department of the Privy Council in a study of outbreaks of splenic fever and cattle plague, and with Dr Burdon-Sanderson in his researches into immunity of cattle against anthrax. Later, with a grant from the British Medical Association, he inquired into the mode of spread of rabies. Mr Banham took part in Dr Greenfield's development of a vaccine against anthrax; and Mr Ingram in Dr Twort's discovery of an essential nutrient substance for the growth of Johne's bacillus. Mr Batt, who was appointed in 1883, proved a valuable assistant to many of the workers in the Institution during the long years he spent at the Brown.

It is difficult to be certain from the existing records exactly how many veterinary assistants there were altogether, when they were appointed, or how long they stayed. During the seventy years of its activity there were at least ten, indicating that their average stay was about seven years; but there may well have been more, as some of them stayed for only a short time. So far as they are known, their names and length of stay are given at the end of this chapter. As already mentioned in Chapter 5, one of them, Mr A. M. Porteous, absconded with a sum of over £100, which had to be written off as a bad debt.

From the first, the Institution seems to have been very popular in the surrounding neighbourhood. It provided a much-needed want, and amply justified the Testator's foresight. The number of animals treated, as distinct from the number of visits paid, was usually about 4000 a year for the first 20 years. After this it tended to rise, reaching a maximum of nearly 8000 by 1910. During the First World War it fell off steadily to 2500 by 1918, but rose again between 1919 and 1924 to 5000–6000. After this second peak it declined to a minimum of 1000 at the outbreak of the Second World War in 1939. The reasons for this were multiple, but the two chief ones were the replacement of houses in the neighbourhood by flats in which the keeping of animals was not allowed, and the springing up of other centres to which sick animals could be taken.

In the first ten years or so 70 % of the animals treated were horses, but this proportion gradually fell till in 1919 it was only 7 %. During the same period the proportion of dogs rose from 20 % in 1874 to 63 % in 1912, after which it fell to 45 %. Cats were not referred to specially in the early records; they were included in the 10 % of other animals. In 1879 they constituted 3 % of the total patients. After this their number rose to 20 % in 1912 and to 42 % in 1919, thus equalling the proportion of dogs. During the twenty years between the First and Second World Wars the nature of the animals is not specified. Most of them were probably cats and dogs, though in 1919 mention is made of 4 % of poultry and other birds.

Most of the animals were treated in the dispensary. A much smaller number, not as a rule more than 4 %, were admitted to hospital as inpatients. This proportion would have been much greater had the accommodation been larger, and had the owners of the animals been able to pay for their upkeep. Animals whose treatment would have required a long stay in hospital often had to be slaughtered, as there was no money available to cover the cost of their maintenance. On one occasion the Senate made a grant of £50 to cover the cost of some long-stay animals, but the continuous financial support that was needed was not forthcoming.

The ills from which the animals suffered varied with the nature of the animal and the prevalence of the different infectious diseases throughout the years. Among horses, lameness was the commonest ailment. Fractures of the leg bones were frequent, often demanding operative treatment. Glanders and farcy were diagnosed in the early years of the Institution, but gradually disappeared after preventive measures had been laid down in an Order of the Board of Agriculture in 1892, and still more so after the passing of the Glanders or Farcy Order of 1907. Before the Muzzling Order came into force in London in 1885 rabies was not uncommon among dogs. In that year no fewer than 26 deaths occurred among human beings in London alone. After, however, the Muzzling Order was extended to the whole country in 1889, accompanied by the destruction of stray dogs, cases of rabies ceased to occur. During 1886–7 investigations were made into an outbreak of rabies among the deer in Richmond Park. Distemper was common among dogs, varying in frequency from year to year. Actinomyces in cattle was detected for the first time in England in 1882. In one year, 1893, there were 504 cases of scabies and 157 of faecal impaction. Most of the animals admitted to

hospital were suffering from injuries. Operations were mainly for tumours, castration and firing (cauterizing).

Many of the animals – horses in particular – were brought to the dispensary in a dreadful state, suffering, as Dr Roy reported, from barbarous neglect or still more barbarous treatment. The nature of the ailment was not always recognized, and Dr Roy, the Superintendent, endeavoured to carry out a post-mortem examination on all animals dying or brought in dead. In 1882 the Veterinary Surgeons Act became law. Dr Roy's comment was that this should have the effect of our seeing 'the last of those ignorant and often barbarous practitioners who, by treatment often worse than actual neglect, have contributed not a little to increase the sufferings of the domesticated lower animals'.

Early in the First World War the hospital had to be closed down because Mr Craig, the Veterinary Assistant, left to join the Army. Two stablemen were discharged, and the caretaker – an ex-Sergeant-Major Farrier – was deputed to deal with minor ailments among the outpatients. At the beginning of the Second World War the hospital had to be closed again for a similar reason. In November 1940 the Institution was damaged by enemy action; the caretaker and laboratory assistant died; the cleaner was evacuated; and the only person residing on the premises was the acting caretaker, Mrs Franklin. From then on the dispensary ceased to function, and for all practical purposes came to an end. The Institution had been active for just on 70 years. During that time it had rendered valuable service to suffering animals and, as will appear in subsequent chapters, had carried out original investigations of great merit into the workings of the body in both health and disease.

So far as they are known, the Veterinary Assistants were as follows:

1871–77	Mr William Duguid, MRCVS
1877–80	Mr George Banham, MRCVS
1880–82	Professor W. F. Garside, MRCVS
1883–1904	Mr Ernest E. Batt, MRCVS
1904–6	Mr A. M. Porteous, MRCVS
1906–9	Mr Malcolm Allan, MRCVS
1910–12	Mr G. L. Y. Ingram, MRCVS
1912–14	Mr Thomas Craig, MRCVS
1920–32	Captain H. C. Driver, MRCVS
1933–39	Mr C. E. Woodrow, MRCVS

## CHAPTER 7: RESEARCH WORK AT THE INSTITUTION

### I MICROBIOLOGICAL

In this chapter and in the three following chapters an account is given of some of the work carried out by the staff and visitors at the Brown Institution. It would be impossible within the compass of these chapters to describe all the research investigations that were undertaken. Only those of special interest for one reason or another have been included, but an account even of these must necessarily be brief. With such a wide range of subjects no classification can be wholly

satisfactory, and the one chosen here is admittedly empirical. It follows that some subjects could be dealt with equally well under either of two headings, and the choice of which one to select has therefore been arbitrary. For example, the prevention of anthrax by vaccination is described under Microbiological Investigations rather than under Diseases of Animals, because the work was concerned more with the anthrax bacillus than with the disease itself. Moreover, no attempt has been made to observe a chronological sequence; and the time at which various researches were undertaken, though often mentioned, is seldom referred to specifically unless a question of priority is concerned.

#### *Microbiological researches*

##### *Diphtheria*

I shall start this chapter by recording one of those dramatic incidents that medicine occasionally affords. The production in small animals of antitoxic serum to diphtheria was described in 1890 by Dr Emil Behring in Germany. Its potential value for curative purposes in man was soon realized. Larger animals were used for its production; and Dr Paul Ehrlich imparted to Behring a method for obtaining a high concentration of antitoxin by regularly increasing doses of toxic cultures of the diphtheria bacillus. Dr Émile Roux in France started to immunize horses in 1892, and with the co-operation of clinical specialists arranged what we should now call a controlled trial of the therapeutic value of the serum, the result of which was conclusively in its favour. In England Dr Armand Ruffer (later Sir Armand), Secretary of the British Institute of Preventive Medicine, after visiting Roux in Paris, took up the preparation of antidiphtheritic serum, benefiting in this task by French experience. At the time, in 1894, Ruffer and his colleague Robertson were working at the Brown Institution, and it was there that the first horse, called Tommy, to be injected in England with diphtheria toxin was stabled. The horse had received only a few injections when one Saturday evening Dr Charles Sherrington (later Sir Charles), Professor-Superintendent of the Brown, received a telegram from his brother-in-law in Sussex saying that his son, George, a boy of seven, had diphtheria, and asking him to come at once. Sherrington, doubting whether the antitoxic titre of the horse would be high enough, took a cab to find Ruffer. Ruffer was dining out, but Sherrington followed up the trail till he found him. Ruffer's words were: 'By all means you can use the horse, but it is not yet ripe for trial.' Sherrington returned to the Brown, and by lantern light bled the horse into a 2-litre sterile flask plugged with cotton-wool. The blood was left on ice to settle. After sterilizing a few smaller flasks, and some pipettes and syringes, Sherrington went home, but returned at midnight to decant the serum. The next morning he took the train to Lewes, where he found Dr Fawsett, a local practitioner, waiting for him at the station in a dog-cart. Dr Fawsett was silent as the awkward package of glassware was packed in, but after Sherrington had climbed up beside him, Fawsett turned to him and said: 'You can do what you like with the boy. He will not be alive at tea-time.' They drove to the Georgian house of the brother-in-law situated under the chalk downs three miles from Lewes. It was a bright frosty morning, but 'tragedy was over

the place, the servants scared and silent'. The boy was very weak, breathing with difficulty, and apparently unable to recognize his uncle. Together, Sherrington and Fawsett injected the serum. The syringes were small, and had to be refilled over and over again. Dr Fawsett left, while Sherrington sat with the boy. Early in the afternoon the boy seemed to be clearly better, and at 3 o'clock Sherrington sent a messenger to Dr Fawsett to say so. Thenceforward progress was uninterrupted. On Tuesday Sherrington returned to London and sought out Ruffer, who immediately said that they must tell Lister (later Lord Lister) about it. Lister, who had some continental visitors to dinner, similarly remarked: 'You must tell my guests about it.' On his insisting, he told them the story in the drawing-room at Park Crescent. The boy suffered from severe paralysis for a time, but later grew to 6 feet in height and held a commission in the First World War.

This story has been told several times. The present account is compiled partly from Dr Claude Dolman's (1973) Donald T. Fraser Memorial Lecture, partly from Sir Alan Drury's description (1948), partly from the Minutes of the Senate of London University, and partly from Sherrington's own written description (1948). (See also Cohen 1958.)

*Bacterial metabolism: growth of the leprosy bacillus and of Johne's bacillus*

*The leprosy bacillus.* In 1909, when Dr F. W. Twort was appointed Superintendent of the Brown, little was known about the chemistry of bacterial growth. Many pathogenic organisms had been cultivated in the laboratory but the nutrient media employed had almost all been selected on empirical grounds. What particular nutrients were required by different organisms, and what the organisms did with them, were questions that had received little attention. That they could be grown and therefore used for purposes of diagnosis and vaccine production was to most bacteriologists all that really mattered. In spite of success with most of the common pathogenic bacteria, two organisms responsible for well-known diseases in man and cattle, namely the human leprosy bacillus and the causative bacillus of Johne's disease of cattle, had hitherto resisted cultivation *in vitro*, even though microscopically they could be demonstrated in large numbers in the tissues. Both of these organisms were acid-fast bacilli closely allied to the human tubercle bacillus. This latter bacillus could be grown on a simple egg medium, but neither the leprosy nor Johne's bacillus could. Twort (1910) argued that the leprosy bacillus required some nutrient substance that the tubercle bacillus was able to synthesize from ordinary media; and that, if dead tubercle bacilli were added to a medium such as Dorset's egg, they would provide the leprosy bacillus with the necessary substance pre-formed. This supposition proved to be correct. Steamed ground-up tubercle bacilli were added in 1% concentration to a glycerine egg medium dispensed in test-tubes. The nasal discharge from a leprosy patient was treated with a 2% solution of ericolin to destroy contaminating organisms and then inoculated onto the medium. After about 6 weeks' incubation at 38 °C a film of growth was first detectable by the naked eye that proved to consist of acid-fast bacilli. Subcultures, however, failed to grow. Whether these organisms were true leprosy bacilli or not is open to question. The probability is that they

were, but that they required something else than the substance provided by the tubercle bacilli for continued growth. It may be surmised that this other substance was contained in just sufficient amount in the original nasal washings to enable a first generation culture to grow, but not a subculture. Be that as it may, what was really important was, as Fildes (1951) pointed out, the demonstration that an organism could grow only when supplied with a substance elaborated by another. 'This is, of course the essential feature of all growth factor work and the basis of all studies of bacterial nutrition.'

Twort's paper had no effect on contemporary thought, and it was not till over twenty years later when growth factors began to be systematically studied that Knight (1936) called attention to the fact that current results were in essentials identical with those of Twort. The reason for the neglect of Twort's paper is, I suspect, that Twort was only one of many workers in different parts of the world who had claimed to have cultivated the leprosy bacillus, and his paper contributed nothing new in this respect. Had he been working with some organism that had not the same emotional effect as had the cause of a world-wide fearsome disease, his paper might have had a different reception. Twort never lost his interest in this subject, but his further attempts to subculture the leprosy bacillus, and the rat leprosy bacillus which closely resembles the human type, proved no more successful than at first.

*Johne's bacillus.* Johnne's bacillus, referred to by Twort & Ingram (1912, 1914) as *Mycobacterium enteritidis chronicae pseudotuberculosis bovis* and now known as *Mycobacterium johnei* or *Mycobacterium paratuberculosis*, is responsible for a chronic inflammatory disease of the intestine of cattle and, less commonly, sheep. Like the leprosy bacillus it belongs to the group of acid-fast organisms classed in the genus *Mycobacterium*, and like the leprosy bacillus it cannot be grown in primary culture without the addition to the medium of an extract of human tubercle bacilli or of some other mycobacterial species such as *Mycobacterium phlei*. Unlike the leprosy bacillus, however, it can be successfully subcultured on this fortified medium, and eventually may even be brought to grow on a synthetic medium to which no mycobacterial extract has been added.

Twort & Ingram's (1912) first paper confirmed the truth of the concept that the study of the leprosy bacillus had introduced into bacteriology, namely that of an 'essential substance' or 'growth factor' necessary for the reproduction of a particular organism. With the help of this substance they isolated Johnne's bacillus, and with pure cultures reproduced the disease in cattle and recovered the bacillus from the experimental lesions.

Though Twort continued to work on the nature of the essential substance, he made little progress because of the lack of biochemical knowledge at the time. Little further was learnt about it till Woolley & McCarter (1940) in the United States concentrated it from a phlei extract by repeated extraction with boiling acetone and obtained an oil that was dissolved in ether and subsequently extracted four times with water. It was not, however, till 1949 that Francis and his colleagues (1949) succeeded in isolating the growth factor in a crystalline state from an aluminium complex that was formed adventitiously on a column of



chromatographic alumina, and not till 1953 that it was freed from the aluminium complex and obtained as a pure substance suitable for chemical determination of its main properties (Francis *et al.* 1953). The substance was given the name 'mycobactin' and was later found to be a complex molecule having five asymmetrical centres (Snow, 1965, 1970).

It is of interest to note that phthiocol, which had been isolated from acid-fast bacteria and possesses the antihæmorrhagic activity of vitamin K, promoted the growth of Johne's bacillus, though not so actively as did a phlei extract. According to Woolley & McCarter (1940), almost without exception bacterial growth factors have been found to be water-soluble compounds. The demonstration that both mycobactin and phthiocol are soluble in fat solvents as well as in water shows that fat-soluble vitamins also play a part in the metabolism of certain bacteria.

#### *Bacterial degradation of creatin and histidine in the intestine*

Interest in the breakdown of nitrogenous substances in the human intestine is of comparatively recent date. Metchnikoff, of course, in the last century, and numerous of his followers had considered the possibility of toxic products of bacterial metabolism being formed in the intestine. Metchnikoff ascribed this rôle to the so-called putrefactive bacteria, but neither he nor his followers was able to identify any specific toxic product. The attempted replacement of these bacteria by the lactic-acid-producing organisms proved disappointing in both its technical accomplishment and its results on human health.

At the Brown Institution Edward Mellanby and F. W. Twort studied the breakdown of creatin and histidine. Barger & Dale (1911) had shown that  $\beta$ -iminazolethylamine – a base produced by splitting off CO<sub>2</sub> from histidine – was present in the intestinal wall, and was largely responsible for the physiological properties of extracts of the intestinal mucosa. They thought that it was a normal product of the mucosa, but Mellanby & Twort (1912–13) brought evidence to show that it was formed by bacterial action in an alkaline medium. This base is among the most noxious substances found in the intestine, giving rise to severe vomiting and purging in cats injected intravenously; and in herbivores, such as the guinea-pig, to constriction of the plain muscle of the bronchi, resulting sometimes in death by asphyxia. Under natural conditions it is probably rendered innocuous in the liver. The identity of the histidine-destroying bacteria was not established, but they appeared to belong to the colon group of organisms.

Twort & Mellanby (1912) likewise described the breakdown of creatin by bacteria in the gut. Twort devised a method of isolating these organisms from the mixture of different species that constituted the intestinal flora. He found that in a creatin meat medium colon bacilli partly destroyed the creatin, but that the most active bacterium in this respect was a Gram-positive obligatory anaerobe closely resembling *Clostridium perfringens*.

*Vaccination against anthrax*

In the middle of last century anthrax (splenic fever, *sang de rate*, Milzbrand) was very prevalent among cattle and sheep in France, Italy and Southern Europe. It was a severe, usually fatal, disease and was the cause of great economic loss. In 1823 Barthelémy showed that it was transmissible to animals by inoculation. Rayer (1850) made similar observations, and described the presence of small, non-motile, filiform bodies in the blood of sheep dead of the disease. (For references see Hutyra & Marek, 1922.)

In a series of papers Davaine (1863*a, b, c*, 1864) showed that anthrax could be transmitted to animals by the subcutaneous inoculation of infected but not of normal blood, and that the blood of an infected animal taken before invasion with the bacilli was non-infective. In the same year Tiegel & Klebs (see Koch, 1881) found that anthrax blood, when filtered through a clay candle, was deprived of its infectivity, while the deposit on the filter remained active. These observations showed, as conclusively as possible in the absence of cultural support, that anthrax was caused by a living organism which multiplied in the body, invaded the blood stream and produced death by septicaemia. The final cultivation and characterization of this organism, *Bacillus anthracis*, was successfully carried out by Robert Koch in 1876 and described in 1877. From then on the main interest was in prevention of the disease or modification of its severity. Before progress along these lines was possible, something had to be learnt about natural and acquired immunity.

Chauveau (1880*a, b, c*) reported that Algerian sheep possessed a natural immunity 'à un degré plus ou moins marqué' to the injection of blood from an animal infected with anthrax and that this immunity could be communicated to European sheep by crossing them with Algerian sheep. Immunity was not complete. A local reaction in the lymph nodes followed the injection, together with mild systemic symptoms. A fortnight or so after a first injection the animals proved refractory to a second, indicating that a partial natural immunity had been reinforced by an acquired immunity.

Pasteur & Chamberland (1880) noted that cattle, like Algerian sheep, were partly refractory to experimental anthrax; they suffered from fever and lymphatic engorgement but did not usually die.

A year before this, namely in 1878, Dr Burdon-Sanderson (later Sir John) and Mr Duguid, his veterinary assistant at the Brown Institution, had found that the inoculation of bovine animals with blood from infected rodents caused a severe but not fatal disease, after which they were partly resistant to reinoculation.

Dr W. S. Greenfield (1880*a*), who succeeded Burdon-Sanderson at the Brown, confirmed these findings, and then went on to modify the virulence of the anthrax bacillus by serial cultivation in aqueous humour, contained in a glass tube sealed after inoculation at both ends and incubated at 35 °C (1881*a*). A steer injected with a third-generation culture suffered from a severe attack of anthrax but recovered in about 10 days. Some weeks later when this animal was re-inoculated with material from the spleen of an infected guinea-pig it suffered from only a

mild illness and was well again in two days. Two further re-inoculations caused no disturbance whatever. It was clear that the animal had become immune as the result of vaccination with a slightly attenuated culture followed by fully virulent organisms.

In a note communicated on 17 June 1880 to the Royal Society of London Greenfield (1880*b*) stated that, when the anthrax bacillus was grown for successive generations in a suitable nutrient fluid it maintained its 'morbific properties' for a number of generations, but each successive generation became less virulent till eventually, after continuous diminution of its virulence, the bacillus, though retaining its morphological characters and its power of growth, became completely innocuous even to the most susceptible class of animals. Thus mice, which fell into this class, were found to be refractory to the inoculation of a 19th generation subculture. The diminution in virulence was evident even at the 8th generation; and no culture later than the 12th generation gave rise to any symptoms. Cattle inoculated with an 8th-generation culture were protected against any serious illness caused by the inoculation of virulent anthrax bacilli. This protection lasted for at least five months, and was effective against both experimental injection and natural exposure to infection on heavily contaminated pasture land (Greenfield 1880*c*, 1881*a-e*).

In July 1880 Toussaint (1880*a*) in France, in a paper communicated to the Académie des Sciences, claimed to have rendered dogs and sheep refractory to experimental anthrax by a preparation the details of which were not disclosed. They were, however, contained in a paper handed to the Academy in a sealed envelope on 12 July. Under pressure, Toussaint consented to the opening of this envelope, and on 2 August the paper was made public. In it Toussaint (1880*b*) described how at first he had used the filtered defibrinated blood of an animal dying of anthrax; but that when he had found this method to be unreliable he had changed to heating the defibrinated blood for 10 minutes at 55 °C. This vaccine rendered sheep refractory to re-inoculation with virulent blood.

Greenfield's papers were published two months or more before Toussaint's (1880*b*) and a year or more before those of Pasteur, Chamberland & Roux (1881*a,b*). It may be noted further that Greenfield's method of attenuating the anthrax bacillus by a process of successive subcultivation was published even before Pasteur (1880) described a similar method for attenuating the virulence of the fowl cholera bacillus.

In his inaugural professorial address at the University of Edinburgh in 1881, Greenfield (1881*c*) described the observations of Burdon-Sanderson and Mr Duguid in 1878 on immunity to anthrax (see p. 346). He then gave an account of his own findings, namely that the inoculation of anthrax bacilli which had lost their virulence by repeated subculture *in vitro* conferred protection lasting for at least five months against subsequent infection with highly virulent material. In his address he said: 'And although I venture to claim for England whatever merit may be due to priority of the discovery, I none the less rejoice that the facts should have been so fully established in France. My experiments were made with a small and inadequate sum of money furnished by the generosity of a private

Society, and in the face of all the difficulties interposed by law; whilst M. Pasteur is encouraged and abundantly supplied with means by the liberality of the French Government.'

For drawing my attention to Greenfield's work I am indebted to Sir Frank Young, F.R.S., of Cambridge University. He told me that it was Brigadier General W. D. Tigertt, M.D., of the United States Army Corps, who had insisted that the credit for first describing the preparation of an effective vaccine against anthrax should be given to Greenfield rather than to Pasteur, whose results he anticipated by a whole year. Going fully into the history and the dating of the various stages in the development of anthrax vaccine in this country and in France, I have been able to confirm the opinion expressed by Brigadier General Tigertt, and am duly grateful to Sir Frank Young for passing this information on to me. I am also grateful to Brigadier General Tigertt himself for kindly giving me an unpublished account of his researches into the subject.

### *The bacteriophage*

From an academic point of view the outstanding contribution to the study of microbiology made at the Brown Institution was the discovery by Dr F. W. Twort of the transmissible lytic agent, later known as the bacteriophage. No other discovery opened up such a wide field of investigation or had such an impact on what is now known as molecular biology.

Twort's first observations were made in 1914 and 1915 with a grant from the Local Government Board and were published in a paper in the *Lancet* at the end of the latter year. During a search for non-pathogenic viruses, which he thought would probably be easier to cultivate than pathogenic ones, he observed that colonies of micrococci developing on various media inoculated with vaccine lymph underwent after a few days a curious change. On egg media the colonies became dull; and on agar slopes they assumed a translucent or transparent appearance first visible at the margin in the form of clear spots. Microscopically the glassy areas consisted of granules staining red with Giemsa. Some of these colonies could not be subcultured; others became transparent. If a portion of one of these glassy areas was used to touch normal colonies of white or yellow micrococci from vaccinia, the colonies became transparent at the point at which they were touched. The transparent growth, when diluted as much as a million times with water or saline and passed through the finest bacterial filters, proved capable of reproducing the change. When, for instance, a single drop of the filtrate was pipetted over a young culture on an agar slope, the growth became dotted with transparent spots that rapidly extended over practically the whole culture. This 'disease' of the micrococcus could be conveyed to fresh cultures for an indefinite number of generations. The transparent material would not grow by itself on any medium, though it retained its activity for over six months in an agar tube. It had little or no action on dead bacteria, and it was inactivated by heating to 60 °C for one hour. Twort was non-committal on the nature of this transmissible lytic agent. He regarded it more as an enzyme-like agent produced by the micrococcus than as a distinct virus. After controversy lasting for several

years it was shown that this view was wrong, and that the lytic agent was, in fact, a filtrable virus that invaded the bacterial cell, multiplied in it, and within half an hour or so produced at the expense of the host such a large progeny of granular particles that the cell burst, liberating these young virus particles, which were then free to invade fresh cells.

Another observation made by Twort was that, if an affected micrococcal culture was repeatedly plated out, a colony from the last subculture might grow normally for months, but eventually undergo the original transparent change. The lytic agent had apparently been carried in what appeared to be a perfectly healthy culture, and then for some reason or other broken through, as it were, and lysed the growing organisms. In modern parlance the phage that can multiply only by the lytic cycle is known as *virulent*; whereas the phage that can persist in the bacterial cell during successive generations without causing lysis is known as *temperate*.

Twort's *Lancet* paper was essentially a preliminary one. Before he could explore the phenomenon of transmissible lysis more fully, he was sent to Salonica to open a base laboratory for the diagnosis of the various enteric diseases that were prevalent among the troops. By the time he returned he found that d'Herelle, a Canadian microbiologist, had independently discovered the lytic agent and published his findings in 1917. He maintained that the lytic agent, later called by him the bacteriophage, was a virus which was parasitic on bacterial cells. The phenomenon of transmissible bacterial lysis is now referred to as the Twort–d'Herelle phenomenon.

When Twort was demobilized at the end of the war he did not pursue, as might have been expected, his early investigations. The reasons for this are not altogether clear, but the lack of financial support from the Local Government Board and the need to reorganize the Brown Institution probably played a major part in his decision. Another, and perhaps equally powerful reason, was the fact that Twort had a very original mind and was bent on exploring unknown territory. Having discovered the lytic agent, and opened up a new field, he was content to leave its tilling to others. By the time he was ready to start work again, d'Herelle (1921) had already published a monograph on the subject recording a more extensive and detailed series of observations than Twort had described in his preliminary paper. Twort, it is true, took part in a discussion of the subject at a meeting of the British Medical Association (Twort, 1922*a*), and published three further papers (1922*b*, 1925, 1931), but none of these communications added anything new. The essential facts about the transmissible lytic agent were contained in his 1915 paper, and on this his reputation must rest.

#### *Other microbiological investigations*

Numerous other microbiological investigations were carried out at the Brown, but space forbids more than a bare mention of a few of them.

It is interesting to note that Dr Cash in 1886 studied the destruction of pathogenic by non-pathogenic bacteria. Anthrax bacilli, for example, in fluid culture, were found to be killed within 3 h by the hay bacillus. This constituted one of the earliest observations on the mutual antagonism of bacteria.

Dr Nathan Raw spent many years studying the loss in virulence of tubercle bacilli. The live vaccine of attenuated bacilli that he prepared was injected in 1932 into more than 8000 calves, and was also used for the immunization of children of tuberculous parents. The protective value of this vaccine, like that of the similar vaccine (B.C.G.) prepared by Calmette and Guérin in France, was not determined at the time, because no controlled observations were made; and by the time they were, the B.C.G. vaccine had monopolized the field. Mr Sibley (1889), working on tuberculosis in reptiles, isolated an acid-fast bacillus, differing from *Mycobacterium tuberculosis*, and known generally as belonging to the cold-blooded type.

Dr Charles Sherrington (later Sir Charles) made a representative collection of bacterial cultures – the forerunner of the national collections that now provide such a valuable service to microbiologists. He also, in his early bacteriological phase in 1891, engaged in the diagnostic cultivation of the bacillus of glanders – a disease very prevalent among horses before the Glanders or Farcy Order of 1907.

Mr Duguid, Victor Horsely (later Sir Victor) and Dr Gerland of Blackburn worked on the rabies virus; Monckton Copeman on variola and vaccinia; C. C. Twort on the serological diagnosis of Johne's disease; Edward Mellanby (later Sir Edward) on infantile diarrhoea and dysentery; Dr Watson Cheyne (later Sir Watson) on the relative dosage of pathogenic organisms; and F. W. Twort on variation and mutation in bacteria, and on the effect of electromagnetic waves on filter-passing viruses.

In the more limited field of parasitology Surgeon Evans in 1890 studied *Plasmodium malariae*, Dr W. Nicoll *Ankylostoma caninum*; and C. C. Twort, coccidioidosis.

#### CHAPTER 7

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