Joseph Lister (1827–1912) acquired a lifelong interest in histology and experimental physiology while a student at University College London between 1848 and 1852. His first two publications in 1853 were histological studies of the contractile tissue of the iris and the skin. Studies of inflammation in 1855 progressed to experiments on the nervous control of arteries, using techniques of peripheral nerve division, spinal cord section and needle stimulation of the brain. This interest in nervous mechanisms led to innovative experiments on gut motility and the autonomic nervous system, from which he inferred that sympathetic nerve control was mediated via intrinsic neuronal plexuses in the gut wall, a mode of action confirmed 100 years later, in 1964–65. It is not generally known that Lister was elected FRS for this early experimental work and that his early commitment to experimental science and microscopy was the background to his later work on the development of surgical antisepsis.

**Keywords:** Joseph Lister; histology and physiology; inflammation; innervation of bowel; sympathetic nerves; anti-vivisectionists

From early childhood Joseph Lister showed a precocious talent for observation and drawing, well illustrated by his drawing of dissections and osteology. His father, Joseph Jackson Lister, was a skilled microscopist who developed the achromatic lens, which provided the great technical advance for the future development of bacteriology and for which he was elected a Fellow of the Royal Society in 1832. Lister senior provided his son with excellent technology and encouraged him to develop the skills in microscopy that were to power much of his early work in experimental physiology as well as his later clinical studies on sepsis and antisepsis. In addition to his father, two of Lister’s teachers at University College London were to have a profound influence on his future career: Thomas Wharton Jones, Professor of Ophthalmic Medicine and Surgery, and William Sharpey, Professor of Anatomy and Physiology.

Wharton Jones had studied the circulation and effects of inflammation by using the translucent tissues of frog webs and bat wings for his microscopic observations on peripheral blood vessels, work that was reported in clear and concise language and
provided a model for Lister’s later investigations of blood flow and inflammation. Sharpey, one of the founders of modern physiology in Britain, was possibly more influential on the young Lister, who in later life acknowledged: ‘As a student at University College I was greatly attracted by Dr Sharpey’s lectures which inspired me with a love of physiology that has never left me.’ Sharpey had studied medicine in Edinburgh, where he became a lifelong friend of the Edinburgh surgeon Professor James Syme. Between 1821 and 1823 Sharpey had studied under Dupuytren and Lisfranc in Paris, and although he gained the Fellowship of the Royal College of Surgeons of Edinburgh in 1830 he decided to base his career on the study of anatomy and physiology. In 1836 he was appointed to the Chair of Anatomy and Physiology at University College London and three years later was elected FRS. In 1853 Sharpey was instrumental in recommending Lister to Syme as his surgical assistant in Edinburgh, where most of Lister’s experimental physiological studies would be performed in the years between 1853 and 1859, before his move to Glasgow to take up the Regius Chair of Surgery. The first two publications were histological studies of the contractile tissue of the iris and the muscular tissue of the skin. A period of intense experimentation resulted in 11 physiological papers between 1857 and 1859, based on extensive and wide ranging research activity, which included studies on the nervous control of arteries, the early stages of inflammation, the structure of nerve fibres, and a remarkable sequence of experiments on the nervous control of the gut, with particular reference to the action of ‘inhibitory’ or sympathetic nerves.

Throughout his life Lister believed that the papers on the microscopy and physiology of inflammation that he presented to the Royal Society in 1857 were his most important publications. His initial observations on the vascular responses in the frog foot were reinforced by studies on the wing of the warm-blooded bat. In 1905, when he was 78 years of age, he wrote: ‘If my works are read when I am gone, these will be the ones most highly thought of’, and in the Huxley Lecture of 1900 it was these particular studies that he detailed in relation to his clinical work on the causation of inflammation and suppuration. Lister’s physiological investigations were meticulous. Edward Sharpey-Schafer, Professor of Physiology at Edinburgh University, later observed that the accuracy that Lister had brought to his observations in physiology and microscopic anatomy had stood him in good stead in his later work in revolutionizing the practice of surgery, and showed the value of a training in physiology to the practical surgeon.

Lister was acutely aware of contemporary advances in physiological research in France, Germany and other countries in Europe, and he frequently discussed observations and results with leading investigators such as Albert von Kölliker, Wilhelm von Wittich, Theodor Schwann and Rudolf Virchow. He was meticulous in referring to the work of other investigators, and he tested their observations and hypotheses with a series of experiments of his own. His fundamental observations on the nervous control of blood vessels, for example, led to a partial disagreement with Eduard Pflüger, who in 1857 had concluded that the splanchnic nerves supplied specific inhibitory fibres to the muscle of the gut wall (Hemmungs-Nervensystem). Lister established a series of experiments to investigate the innervation of the gut for himself, from which he published many accurate and original observations, and in which he was careful to acknowledge the influence of others. In 1884, for example, 26 years after the publication of his investigations on the function of intestinal sympathetic nerves, he wrote: ‘I happened, I believe, to be the first to use the word “inhibitory” in English physiology, by the advice of my old friend Dr Sharpey, with reference to an early paper I was about to publish on what the Germans term the “Hemmungs-Nervensystem”.’
LISTER’S EARLY PHYSIOLOGICAL RESEARCHES

The publication in 1853 of Lister’s first major research project concerned the microscopic structure and function of the iris. There were at that time opposing views on the presence or absence of separate constrictor and dilator muscle fibres in the iris.

Lister undertook an overview of the existing literature, studied tissue from the horse, cat, rabbit and guinea-pig in addition to six surgical specimens taken from patients during surgical procedures on the eye, and described the structure and arrangement of the iris. His analysis of the observations from earlier workers was masterful: in his description of the microscopic appearance of granules within the muscle cells, Lister gave credit to his former teacher, stating: ‘This tendency to transverse arrangement of the granules was long since noticed by Mr Wharton Jones, as this gentleman has informed me’. In contrast, he was not averse to giving constructive criticism and gently chided the eminent surgeon and physiologist William Bowman for confusing muscle fibres with the walls of blood vessels. The paper reveals thorough and meticulous work, reported with a humility that typified Lister, who stated that his engagements prevented him from carrying the inquiry further, apologizing for offering the results of an ‘incomplete investigation’. His key finding was to demonstrate that the iris is composed of smooth muscle fibres arranged in both constrictor and dilator muscles, correcting the belief of previous workers that there was no specific dilator pupillae muscle.

Lister’s next study, on the muscular tissue of the skin, also appeared in Quarterly Journal of Microscopical Science in 1853. He was able to confirm Albert von Kölliker’s observations that in contrast with other mammals—in which the large tactile hairs (the vibrissae) are associated with striated muscle—in humans, smooth muscle fibres are responsible for the erectile function (horripilation) of hair. His manual dexterity was demonstrated in describing a new method of cutting thin histological sections of the relatively firm tissue of the scalp. Such was the extraordinary skill of Lister’s microscopy that he could gently correct Friedrich Gustav Henle, a researcher regarded as perhaps the greatest German histologist of the nineteenth century, for mistaking small blood vessels for muscle fibres. Both of these histological papers were illustrated with skilful drawings made with the camera lucida, which, Lister affirmed, ‘has the great advantage of ensuring correctness of proportions’.

The histology and function of plain (unstriped) muscle were the subject of Lister’s third paper, on the minute structure of involuntary muscular fibre, which appeared in the same journal in 1857. The work was designed to test Kölliker’s observations on the structure of individual muscle fibres. Lister confirmed the observations in the foot of the frog and extended them to the muscle in the wall of arteries, work he was undertaking in parallel in his study of inflammatory responses. He reported that the muscle fibres of the blood vessels were similar to those that Kölliker had found in pig intestine, but they were coiled spirally and individually around the vessels within the intermediate layer of the wall.

A short report of 1858 concerned the flow of lymph and emulsified fats (chyle) in the mesentery of the mouse intestine. The study had two objectives: to define the character of the flow in the lymphatics, and to investigate the commonly held belief that lacteals in the gut wall could absorb solid matter from the lumen. After anaesthetizing a mouse with chloroform he opened the abdomen and withdrew a loop of intestine on to a glass plate under a microscope and saw mesenteric lymph flowing in a steady stream, without visible

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contractions of the lymph vessels. He noted muscle fibres in the walls of the vessels, which contained valves, but did not report rhythmic contractile activity (it is possible that the chloroform anaesthesia he had employed served to inhibit movements in the lymphatic walls). In the second part of this study Lister fed mice with indigo, a coloured substance consisting of indigestible fat-based molecules. He found that the indigo was not absorbed by the gut, and he expressed ‘great doubt on the possibility of absorption of solid matter by the lacteals.’

Lister published seven papers in 1858 on the results of experimental physiological investigations on the origin and mechanism of inflammation. Two of them concerned the nervous control of blood vessels\textsuperscript{16} and the early stages of inflammation.\textsuperscript{17} They referred to experiments that were planned to investigate a contemporary dispute between physiologists, concerning the origin of the control of blood vessel calibre by the sympathetic nervous system. A series of experiments, in which he observed the diameter of blood vessels in the frog web with an ocular micrometer before and after the ablation of parts of the central nervous system, and before and after division of the sciatic nerve, led him to conclude—in disagreement with Wharton Jones\textsuperscript{2}—that vascular tone in the leg was under the control of the spinal cord and medulla oblongata. These experiments were remarkable for the finesse of technique and for the logic of their planning.

The progression of inflammation to suppuration—and, often, death—was a common and much-feared event after surgery, and gave impetus to Lister’s studies on inflammation and tissue fluid flow. His experiments on the early stages of inflammation were made on the web of the frog foot and the bat wing, developing the work of Wharton Jones. Lister performed investigations of erythrocyte adhesiveness, examining blood taken from his own inflamed distal phalanx and comparing it with blood from a normal finger, and divided his report into four sections:

(i) the aggregation of red blood cells when removed from the body (clotting),
(ii) the structure and function of blood vessels,
(iii) the effects of irritants on blood vessels, and
(iv) the effects of irritants on tissues.

He demonstrated that capillary flow in the frog web was governed by the constriction or dilation of the arteries and was affected by local irritation, trauma, or reflex activity through the central nervous system. He was firm in his observations that the walls of capillaries were devoid of muscle fibres yet highly elastic and capable of great variations in capacity determined by arterial flow into the vascular bed. The vascular reactions to trauma and various irritants were illustrated with superb camera-lucida drawings that illustrated vascular stasis and congestion in an initial nervous response to injury. He pointed out that initial vascular changes were the result of reflexes through the nervous system succeeded by vascular changes secondary to local tissue damage. Rickman Godlee wrote of this paper that it ‘impresses the reader by the beauty and simplicity of the experiments described, the originality of the thoughts, and the soundness of the reasoning.’\textsuperscript{18} In a letter dated 10 April 1859 Lister quoted the eminent neurophysiologist Brown-Séquard, who had spoken of ‘the beautiful researches of Mr Lister, and giving me the full credit of establishing the essentials regarding inflammation.’\textsuperscript{19}

In the conclusion of the paper, Lister related his experimental observations to clinical situations such as skin damage from boiling water and trauma from surgical incisions.
Although the role of infection had yet to be discovered, these early studies of inflammation were of fundamental importance to Lister’s future clinical work on the healing of wounds and the effects of infection on tissues. Godlee reported that ‘the paper was well received at home and on the continent and its conclusions have, with scarcely an exception, stood the test of time.’

Following an observation that the inflammatory process in some forms of septicaemia affects the lining of blood vessels, leading to blood clotting within the vessels, Lister later returned to the subject for his Croonian Lecture at the Royal Society in 1863. Previous theories had suggested that blood remains liquid in the vessels because of the presence of a small amount of ammonia. Lister proved those theories wrong, and from experiments using lengths of animal jugular vein he concluded that damage to the lining of blood vessels was an important cause of intravascular coagulation. He did not have knowledge of the coagulation cascade, but his observations on diseased blood vessels contributed to the present-day understanding of clotting.

Lister’s deep interest in the nervous control of blood vessels led him into an investigation of the nervous control of the gut that concluded with a remarkable inference on the mode of action of sympathetic nerves on gut motor activity, which was not confirmed histologically for a century. His investigations, published in *Proceedings of the Royal Society*, were in the form of a letter addressed to Dr Sharpey, the Secretary of the Society. Lister’s interest in the gut, at a time when he was doing extensive work on the function of blood vessels in inflammation, had been stimulated by Pflüger’s suggestion that the splanchnic nerves supplied the muscle layers of the intestine and contained specific inhibitory fibres, the *Hemmungs-Nervensystem*. It was Sharpey who first suggested that this phrase could be translated as ‘inhibitory nervous system’. Pflüger had proposed the idea of specific inhibitory nerves, but Lister disagreed: he believed that the same nerve fibres were responsible for both increased and decreased muscle activity, depending on the strength of the stimulus applied. This view stemmed from studies of blood vessels in inflamed tissues in which Lister had observed that the arteries in the frog foot constricted after the application of a mild stimulus and relaxed with stronger stimulation, acting—he believed—through the same nerves.

Despite this incorrect hypothesis, in experiments combining both mechanical and electrical nerve stimulation performed in June and July 1858 Lister made an important inference concerning the mode of action of the splanchnic nerves. He chose rabbits, with their very active gut movements, for experiments without chloroform anaesthesia to avoid its depressant effect on gut reflexes. In the first experiment a length of small bowel was allowed to protrude through an incision in the animal’s flank, and electrodes were applied to the visceral nerves at their origin from the spinal cord. Electrical stimulation caused complete relaxation of the gut, but local stimulation of the bowel caused a small localized contraction that did not spread to adjacent bowel. Lister concluded: ‘this observation is of fundamental importance, since it proves that the inhibitory influence does not operate directly upon the muscular tissue, but upon the nervous apparatus by which its contractions are, under ordinary circumstances, elicited.’

In the second experiment he examined the effects of devascularization by ligating the vessels supplying a segment of bowel, a procedure that resulted in increased peristalsis. Stimulation of the sympathetic nerves again caused a relaxation of the gut. Lister again concluded that gut activity was controlled by nerves in the bowel wall that had been stimulated by the loss of blood supply.
In the final experiment he removed the fine nerves to a segment of bowel without damaging the blood supply. Sympathetic nerve stimulation now had no effect on the denervated segment of bowel, which continued to contract spontaneously, allowing Lister to conclude:

the persistence of the vermicular motion after complete division of the mesenteric nerves shows that the movement...is effected by a mechanism within the intestine: and its continuance in the portion of gut so treated, while other parts are relaxed, on the application of galvanism to the spine, proves that the inhibiting effect acts through the mesenteric nerves.

In addition to these experiments, his histological examination of the bowel wall revealed the presence of a plexus of neurons, confirming George Meissner’s observation of the previous year (1857), and concluded with the remarkable inference: ‘it appears that the intestines possess an intrinsic ganglionic apparatus which is in all cases essential to the peristaltic movements, and, while capable of independent action, is liable to be stimulated or checked by other parts of the nervous system.’ Curiously, Lister denied the existence of inhibitory sympathetic nerves and concluded saying:

it is safest in the present state of science to regard as a fundamental truth not yet explained, that one and the same afferent nerve may, according as it is operating mildly or energetically, either exalt or depress the functions of the nervous centre on which it acts. It is, I believe, upon this that all inhibitory influence depends.

His mind seemed to be closed to the specific inhibitory function of the sympathetic nervous system, yet he inferred that extrinsic nerves controlled intestinal motor activity indirectly through their effect on the intramural plexus of neurons. This conclusion was generally ignored, and the belief persisted that inhibitory sympathetic nerves caused relaxation by a direct effect on the muscle fibres of the gut.

It was not until the development of histochemical techniques in the mid twentieth century that Lister’s belief in the effect of extrinsic nerves on the intramural plexus was confirmed, by K. A. Norberg in 1964. Techniques such as formalin-induced fluorescence in adrenergic sympathetic nerves finally demonstrated the synaptic relationship with intrinsic gut neurons (see figure 1). Furthermore, histochemical and physiological investigations of bowel resected from patients born with the condition known as congenital aganglionosis (Hirschsprung’s disease), in which the intrinsic ganglia in the bowel wall are absent from birth, showed that coordinated contractions and relaxations do not occur in such gut in spite of an often dense muscular innervation by sympathetic and parasympathetic nerves. As a result, patients with the condition suffer from chronic intestinal obstruction, something that in principle could have been predicted from Lister’s series of experiments.

CONCLUSIONS

In this paper an attempt has been made to elucidate Lister’s skill as a microscopist and experimental physiologist at a time when the technology available to physiologists was in its infancy, and to show how microscopy provided a crucial component of the scientific basis for his future clinical work on inflammation and sepsis. Many of the conclusions that Lister drew from meticulous early work, aided by his acute powers of observation,
have stood the test of time. The renowned pathologist Cuthbert Dukes had no doubt as to the
stature of this early work, which formed the basis of the later studies on infection and the
introduction of antiseptic surgery, when he wrote: ‘Lister’s papers might be studied with
great profit by those who would devote themselves to experimental work. Throughout his
papers he shows himself to have been an inductive philosopher with a genius for seeing
at once the precise experiment necessary to clear up a doubtful point.’

NOTES
2 T. Wharton Jones, ‘Observations on the state of the blood and the blood vessels in
3 M. Worboys, ‘Joseph Lister and the performance of antiseptic surgery’, Notes Rec. R. Soc. 67
(this issue) (http://dx.doi.org/rsnr.2013.0028).
(1902).
7 Fisher, op. cit. (note 1), p. 89.
16 J. Lister, ‘An inquiry regarding the parts of the nervous system which regulate the contractions of the arteries’, *Phil. Trans. R. Soc. Lond.* 148, 607–625 (1858). [Read at the Royal Society on 18 June 1857.]
20 Godlee, *op. cit.* (note 18), p. 49.
22 J. Lister, ‘Preliminary account of an inquiry into the functions of the visceral nerves, with special reference to the so-called “inhibitory system”’, *Proc. R. Soc. Lond.* 9, 367–380 (1857–59). Many years after these experiments, Lister was asked on Queen Victoria’s behalf for his support for new legislation being planned against vivisection. Lister declined. (See Fisher, *op. cit.* (note 1), p. 218.) He was in fact a doughty supporter of experimentation on animals: in response to a question from Thomas Huxley during the proceedings of the 1875 Royal Commission on the matter, Lister replied: ‘These early experiments had the effect of giving me a kind of pathological information, without which I could not by possibility have made my way in the subject of antisepsis.’ See *Report on the Royal Commission on the practice of subjecting live animals to experiments for scientific purposes*, p. 215 (Eyre & Spottiswoode, London, 1876).