"A Hypothesis Dispossessed by New Facts Dies an honourable death..." The Hunterian Method and its Legacy to Contemporary Cardiovascular Research

2023 has brought with it the opening of the beautifully refurbished Hunterian Museum – visiting has been a wonderful experience and a welcome reminder of the first stirrings of scientific surgery. In addition to its importance in science communication and public engagement, we must remember the collection's original purpose was to illustrate the structural basis for physiological function. It is imperative to conserve the links between Hunter's physical works and the principles he was using them to illustrate: the museum does a tremendous job of guiding us through these conceptual hoops as well as the wider context in which Hunter was operating and innovating. Hunter's legacy has been extensively examined and lauded but what is perhaps less-commonly discussed is how the lasting impact of his conceptual application of the scientific method to surgery – what has come to be referred to as the "Hunterian Method" – still forms the framework behind much contemporary medical research. It is my conviction that this contribution is far superior in impact to the physical collection alone and thus ultimately the most important element of Hunter's legacy to conserve. This philosophy does not need excavating from the past. To find it we merely have to examine contemporary research where the continued influence of the Hunterian Method is obvious.

The Hunterian Method is more than just the application of the scientific method to surgery. For the first time, Hunter brought together the study of the natural world with human anatomy and physiology to generate hypotheses he would interrogate with the scientific method. An insight that may seem simple to us now only because of its ubiquity in modern practice. Above all, Hunter's emphasis was on trusting the evidence before him over a reliance on the immutable truth of the works of Galen and the other old masters.

Here, I argue that Hunter's method brought together three distinct elements in a manner previously unseen: comparative anatomy, hypothesis testing driven by the scientific method, and careful evidence-based translation into clinical practice and beyond. Hunter broke with convention by blurring the lines between physiology, surgery, and study of the natural world, ironically best summarised by his most notorious critic Jessé Foote, who described Hunter's lectures as "something new – consisting of surgical, physiological, and comparative anatomy branches, and so mixing them together, as to confound or illustrate each other"[1]. Despite Foote's incredulity at the approach, teaching the intertwining basic sciences underlying clinical practice has become the gold-standard approach to medical education – and indeed medical practice – and would be instantly recognisable to the modern medical student.

It would be an impossible task to draw together examples from all scientific disciplines touched by Hunter's influence. Instead, I will focus on modern-day cardiovascular science to illustrate the potency and persistence of combining the elements above: an ideal field to explore his legacy, spanning from bench to bedside to public health. Given Hunter's own demise by myocardial infarction (MI), there is every chance this is where he would be most pleased to have left his legacy. Hunter's methodology so permeates contemporary scientific research that in every modern example presented, the experimental rationale is mirrored in related experiments by Hunter. In the face of ever-improving tools and resolution, the Hunterian Method is conserved.

Comparative Anatomy

The first tenant defining the Hunterian Method – comparative anatomy – is broadly defined as the study of structural similarities and differences between organisms[2], Hunter employed this approach to generate hypotheses of anatomical function in health and disease which could be tested. It should be noted that comparative anatomy did not attract its Darwinian association until the publication of *Origin of Species* in 1859, the century after Hunter's death. While we now often associate this discipline purely with its role in evolutionary biology, consigned to history and superseded by genetic techniques, it is as relevant now as it ever was, merely under a different guise.

Figure 1. Specimen 2212, showing segments of detached Lizard (*Lacerta Spp*) tail. Assorted specimens display other lizards at varying stages of tail regrowth. Collected by Hunter in Portugal. (Author's own photograph. Hunterian Museum, RCS)

During Hunter's time as an army surgeon in Portugal he collected lizards that could readily detach their tails "by the strength of the animal alone" to escape predation, before regrowing it (**Fig.1**)[1][3]. Hunter supposed if their tails could regrow, so might other structures. He thus demonstrated their capacity for limb regeneration and observed the non-scarring nature of their wound sites as compared with non-regenerative animals.

Comparisons between species has not disappeared as an experimental technique. In fact, animals with capacity for regeneration – including salamanders – are used in contemporary cardiovascular research for their ability to perform scarless healing of cardiac tissue following MI; differences in genetics and wound microenvironment between these organisms and non-regenerative mammals are used to identify targets to enhance cardiac repair[4]. A notable exception to the rule of mammal non-regeneration is the spiny mouse, which exhibits highly efficient cardiac repair and also possesses the remarkable ability to shed portions of its skin to escape predation before full regrowth of the dermis and epidermis – much like Hunter's lizards[5].

Comparative anatomy is not constrained to differences between species; Hunter found particular interest in variations between animals of the same species through his study of sex differences and developmental stages. By comparing life stages of the same species, he generated hypotheses regarding developmental origin of structures. This remains a tool in contemporary cardiovascular research, both in foetal and post-natal development. A striking example of changing postnatal phenotype is the capacity of the early neonatal mouse to fully regenerate its heart following MI, an ability lost by postnatal day 7[4]. Comparison between these organisms forms the basis for contemporary regenerative medicine, arguably the future of basic surgical science.

We find continued use for comparative anatomy in transgenic approaches that allow us to move beyond natural phenotypic differences. By comparing transgenic and wildtype animals of the same species, we increase the resolution of the comparison: a smaller degree of difference allows for more precise hypotheses of what is causing the phenotype. Transgenic animals may seem a far-cry from Hunter's work, but the concept would be familiar to him; he was particularly keen to obtain 'monsters' – animals with morphological defects – and compare them with wildtype specimens. For example, in his brilliantly titled *Account of an Extraordinary Pheasant*, he describes a female pheasant exhibiting male plumage due to endocrine dysfunction[6]. Our tools may have evolved beyond his comprehension, but Hunter would surely recognise the principles of comparative anatomy alive and well.

Applying The Scientific Method

Hunter was no mere stamp collector. His great insight was to subject the hypotheses generated from comparative anatomy to the scientific method of the enlightenment, to reject or tentatively accept them on the basis of evidence obtained by experimentation, manipulating individual variables to verify presumptions.

We can illustrate this second principle of the Hunterian Method with Hunter's famous Richmond Stag Experiment, passed down by word-of-mouth[7] and often discussed in reference to his later popliteal aneurysm surgery. The initial hypothesis was grounded in comparative anatomy of the stag antler and human femur, that the antlers were supplied by arteries in a manner analogous to bone. Hunter supposed therefore, that ligation of the artery supplying the antler would provide a model of femoral necrosis, an early attempt to generate an animal model of disease. This hypothesis was "dispossessed by new facts and died an honourable death", to use Hunter's own phrase[8], when survival of the antler following ligation became apparent; dissection revealed collateral vessel growth had facilitated continued supply of the tissue. This discovery was only possible by application of his method. Without his initial hypothesis that the bone would necrose, there would have been no need to perform the ligation, nothing deemed strange about the result, and no discovery of collateral growth. In his day, familiarity with theories of the "vasomotor and sympathetic action" of arteries allowed him to accurately infer that these vessels grew "under the stimulus of necessity": what we might now refer to as hypoxia stimulating angiogenesis and collateral vessel formation.

While antlers still have their place in medical research – most interestingly in my opinion by the use of reindeer velvet as a model of scarless skin regeneration (**Fig.2**)[9] – comparable work in the cardiovascular field can be found in the realm of collateral coronary artery formation. Taking the previous example of the regenerating neonatal mouse heart, Red-Horse and colleagues demonstrated one notable difference at postnatal day 1 vs 7 following MI induction was the significantly increased formation of collaterals (**Fig.3**)[10]. Hunter would have recognised the parallels with his own work, and the resultant benefit to the infarct site. The selective ability of the early neonatal heart to generate extensive collaterals demanded further analysis: comparison between vascular anatomy of knockout and wildtype hearts was conducted to identify a role for the CXCL12 pathway[10]. The philosophy is Hunter's, but the methods refined, and resolution increased.

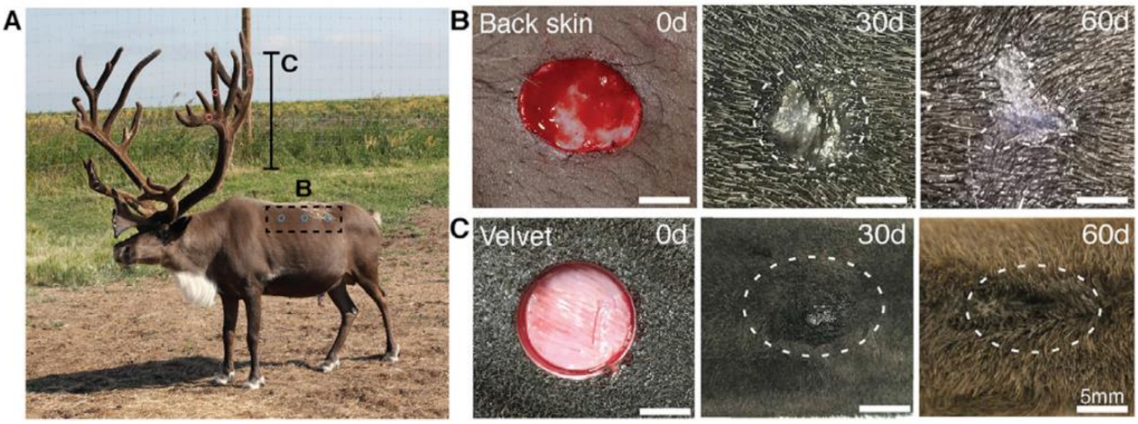

Figure 2. Scarless skin (velvet) regeneration of reindeer antlers[9].

Arterial ligation is not the only technique in Hunter's arsenal still used. His technological advancements in dissection with his brother William facilitated the demonstration that dye entering the canine duodenum is taken up by lacteals and carried into the lymphatics, revealing their structural continuity and responsibility in absorbing the products of digestion (**Fig.4**)[11].

This technique is strikingly similar to cardiovascular work from Klotz and colleagues examining the lymphatic drainage of the murine heart and the benefits following MI[12]. Dye was administered into the myocardial tissue and its drainage to mediastinal lymph nodes confirmed by dissection. They then demonstrated the difference in lymphatic territories in MI vs non-injury hearts and showed exogenous VEGF-C could elicit lymphangiogenesis (**Fig.5**) and improve functional recovery post-MI[12].

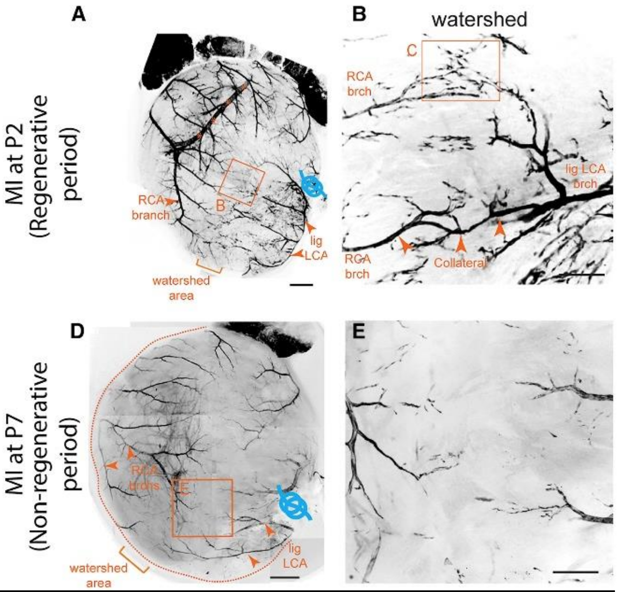

Figure 3. Collateral formation in mouse heart following MI at postnatal day 2 (P2) and postnatal day 7 (P7)[10]

Figure 4. Mercury-filled lymphatics of the small intestine of a turtle.[11]

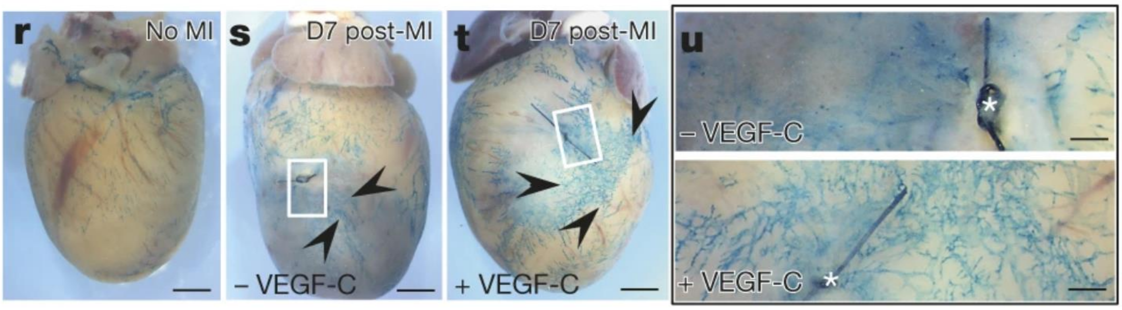

Figure 5. Staining of mouse cardiac lymphatics with dye. Exogenous VEGF-C enhances lymphangiogenesis following MI.[12]

Clinical Translation

Furthering anatomical knowledge and theories was not an end in itself to Hunter, his goal as a surgeon was to apply these findings to improve clinical outcomes for his patients. His work on antler collaterals was famously translated to popliteal aneurysm surgery, his work on lymphatic staining set the stage for later intraoperative identification of lymphatics using dye. Despite these successes he was characteristically reluctant to translate every finding into clinical practice without sufficient evidence: "an operation is to mutilate a patient we cannot cure"[8]. Much of his work reflected this grounding in evidence, not least his work on fertility in pigs – Hunter demonstrated that ovaries are predestined to produce a fixed number of offspring by removing a single ovary to halve litter sizes[13]. What is remarkable about this experiment are the terms in which it is described by Hunter: "the difference in the number of [piglets] produced by each was greater than can be justly imputed to accident." This foreshadowing of modern hypothesis testing makes clear the value Hunter placed on evidence. That his tuition of Jenner is often a footnote in accounts of Hunter's life, despite the impact this had on epidemiology is a testament to the wider achievements of the man.

Significance testing is of clear importance, but so is the use of pigs as a translational milestone in cardiac science, given their hearts are of similar dimension and function to humans. In fact, there are unpublished porcine trials of a VEGF-C analogue ongoing in Bristol on the basis of Klotz's work outlined above. Red-Horse and colleagues have similar translational aims, asking if CXCL12 induces collateral formation in neonates, will exogenous administration do the same in adults? Astonishingly the answer is yes, exogenous administration can act through dormant developmental pathways to induce collateral growth post-MI (**Fig.6**)[10]. Noble though knowledge for its own sake may be, the goal of basic cardiovascular research remains translational, and Hunter would agree that improving the lives of patients should remain the end-goal of any such endeavour.

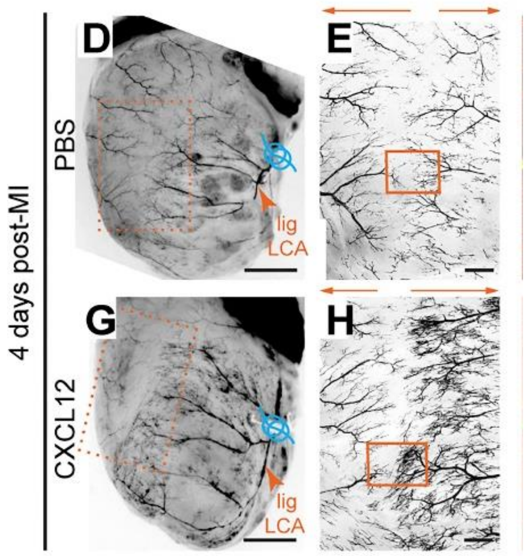

Figure 6. Exogenous CXCL12 enhances collateral artery formation following MI.[10]

Conclusion

I believe it is the case that once the ubiquity of the Hunterian Method is revealed, one cannot help but see it in every contemporary case, article, and paper that is read. Hunter stitched together the very fabric of modern surgical thought to the extent that his revolutionary ideas are now taken as self-evident. His humble beginnings and indefatigable spirit today serve as inspiration to surgeons, physicians, and scientists from all backgrounds and walks of life – I would wager the Hunterian Museum will ensure this remains the case for generations to come.

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