fantastic start by building a scaled-down robotic version of their fossil tail and testing its propulsive capabilities in a water flume. Now, what is needed are whole-body, three-dimensional digital studies testing how an entire animal the size and shape of *Spinosaurus* — with its deep tail and gaudy sail and big arms and stubby legs — could move, hunt, and bite in the water.

Where can I find out more?

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Primer

The glymphatic system

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All biological processes including movement, digestion, and thought require energy, and the metabolic activity necessary to generate that energy also generates waste. Metabolic by-products, including a variety of proteins, accumulate in the tissue and can be harmful if not removed. Throughout the body, the lymphatic system is responsible for clearing metabolic waste from the tissue (Figure 1). An ultrafiltrate of blood plasma constantly enters the tissue from the microvasculature and percolates through the tissue before uptake by lymphatic capillaries. Once in these capillaries, a series of lymphatic vessels, pumps, and valves ensures directional fluid movement, ultimately draining the lymph containing waste products back into the venous circulatory system. The liver can be regarded as the ultimate recycling station in the body, where these waste products are either degraded or reused.

The brain is one of the most energetically demanding organs within the body. However, the presence of the blood–brain barrier limits the influx of the plasma ultrafiltrate from the microvascular bed, which in peripheral tissue drives waste clearance. The brain tissue also lacks traditional lymphatic capillaries to establish directional fluid movement and waste clearance. Instead, the brain uses a network termed the glymphatic system to support a constant influx of cerebrospinal fluid (CSF) that drives the export of metabolic waste.

The glymphatic system is a network of tunnel-like perivascular spaces that promotes directional, bulk fluid movement through the brain (Figure 1). Glymphatic flow consists of CSF entering the periarterial spaces of the brain, penetrating into the tissue and mixing with interstitial fluid (ISF), and ultimately carrying solutes to the perivenous space to exit the brain tissue. This fluid–solute mixture exits the brain via CSF efflux routes from the subarachnoid space, such as those that run alongside cranial nerves, the cribiform plate and the meningeal lymphatic vessels. In this primer, we provide an overview of what is known about the perivascular spaces that make up the glymphatic system, the drivers of brain fluid movement, the role of the glymphatic system during sleep, and the changes to glymphatic function in disease. The goal of this article is to provide a basic understanding of the glymphatic system and identify key questions for future research.

The glymphatic system: Optimized to drive flow

The organization of the brain supports directional glymphatic fluid transport, as exemplified by the highly ordered nature of the perivascular space. Perivascular spaces are fluid-containing channels that run alongside the vasculature, enclosed by the vascular endfeet of astrocytes. The brain vasculature comprises endothelial cells surrounded by smooth muscle cells or pericytes. Astrocytes — glial cells within the brain — ensheath the vasculature with their specialized endfeet processes. These astrocytic endfeet delineate the perivascular space from the brain tissue, creating tunnel-like structures that enable fast fluid flow.

The shape and flow along these perivascular spaces change dramatically in different segments of the vascular network. The periarterial space in the first segments of the major cerebral arteries, also called the Virchow-Robbins space, is wide, and the circular blood vessel sits slightly off-center inside an oblate ellipse of astrocytic endfeet. Modeling estimates of fluid velocity suggest that this shape minimizes hydraulic resistance. Arterial pulsation — contraction and dilation of the vasculature throughout the cardiac cycle — changes the dimensions of the perivascular space, sending a pulse of CSF into the brain and promoting directional fluid movement along the periarterial spaces. Little is known, however, about the perivenous regulation of fluid efflux. Injections of CSF tracer into the cisterna magna of the subarachnoid space and into brain tissue show clearance along the perivenous space in fixed specimens.

However, the primary draining veins are located deep within the brain or at the base of the skull, preventing interrogation with current *in vivo* imaging approaches. Further work is necessary to understand how the perivenous glymphatic system interfaces with the meningeal lymphatic system.

Astrocytes are specialized to regulate both perivascular and interstitial flow. Astrocyte endfeet decrease in size as arterial diameter decreases. Protein complexes such as the dystrophin-associated complex, which anchors the water channel aquaporin 4 (AQP4) at the membrane, are enriched at astrocyte endfeet. Localization of AQP4 at the vascular endfeet is necessary for the development of glymphatic flow, with initial perivascular influx beginning in the circle of Willis, an arterial network at the base of the skull, at mouse embryonic day 17.5 and then established in the cortex by postnatal day 14. Loss of AQP4 polarization on astrocytic endfeet in adult mice leads to a reduction in glymphatic flow. Finally, astrocytes exhibit highly dynamic cell-volume regulation and buffer a variety of interstitial ions such as K^+, which can directly impact the volume and shape of the interstitial space. The contribution of astrocytes to local and global glymphatic fluid flow is an active area of research.

As arteries penetrate the brain, the perivascular space dramatically reduces in size. Histological analysis of post-mortem tissue suggests that, in the case of capillaries, which provide most of the oxygenation to the brain tissue, the perivascular space is smaller and most flow occurs in the interstitial space instead of in the clearly defined channels of the perivascular space. However, existing imaging techniques do not have the depth or resolution to image the capillary perivascular space or the interstitial space *in vivo*. The size and shape of the perivascular space in the microvasculature, and how fluid moves through the interstitial space, are therefore open questions in the field.

Glymphatics and sleep

Most organisms have a sleep-like state in which the brain shows fundamentally different activity patterns and sensory processing compared with the awake state. Yet, the neurobiological purpose of sleep has long eluded scientists. The activation of glymphatic fluid transport during sleep might provide an answer.

Evidence for this hypothesis came from a set of experiments where a fluorescent tracer was injected into the CSF of naturally sleeping or awake mice positioned under a two-photon microscope. When mice were awake, limited amounts of tracer entered the penetrating perivascular spaces or the brain parenchyma. But, when mice were asleep, fluid movement increased by an order of magnitude, with a significant amount entering penetrating arterial perivascular spaces (Figure 2). This effect was accompanied by increased interstitial space volume, suggesting that interstitial fluid movement was also increased. This finding has since been extended to

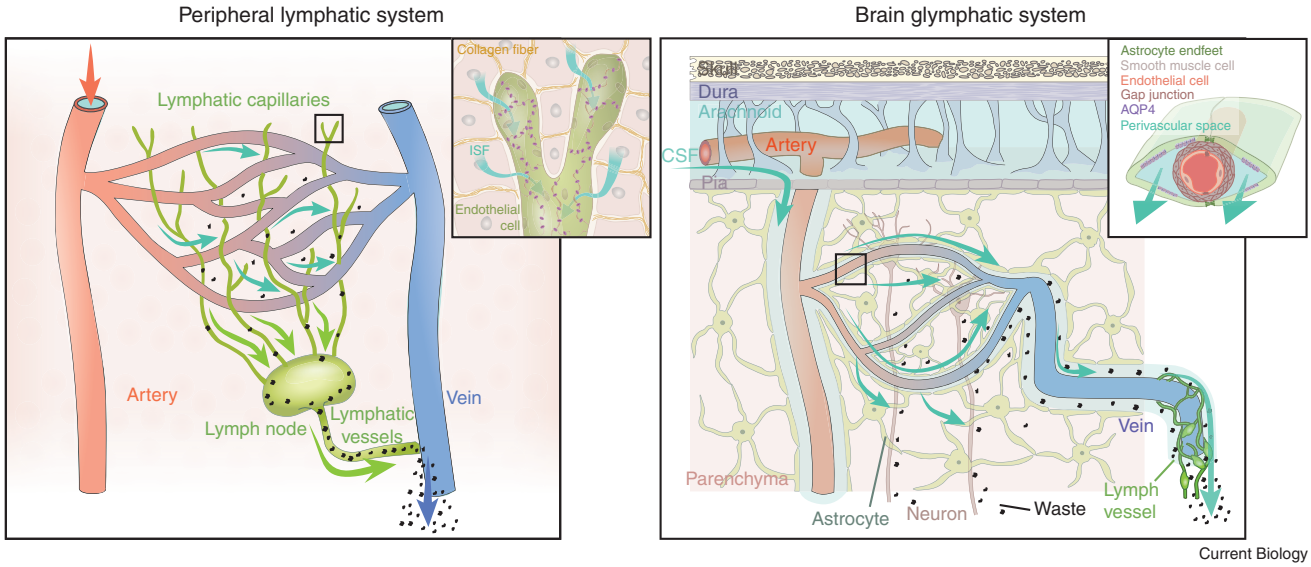

Figure 1. Comparison of peripheral lymphatic and brain glymphatic interstitial fluid movement.
(Left) In the periphery, plasma ultrafiltrate from the microvasculature (light blue arrow) percolates through the tissue and enters lymphatic capillaries (green) enmeshed in the tissue. Once in the capillaries, the interstitial fluid (ISF) is pulled into larger vessels and lymph nodes (green arrows) via vessel contraction and one-way valves. Ultimately, this fluid is returned to the venous system. Dark red and blue arrows indicate blood flow direction. The inset (the black box in the main figure indicates the region of interest) shows the ISF movement (blue) between the endothelial cells (green) of the lymphatic capillaries. These capillaries are attached to the tissue via collagen fibers (brown). (Right) The glymphatic system is the functional analog of the lymphatic system in the brain. Instead of the influx of ultrafiltrate from the capillaries, which is restricted due to the blood–brain barrier, fluid moves along perivascular spaces surrounded by astrocyte end feet. Cerebrospinal fluid (CSF) enters the brain parenchyma alongside the arteries (light blue arrows) and ISF ultimately leaves the brain tissue via perivenous spaces, perineuronal spaces, and lymphatic vasculature in the meninges, where it enters the more traditional lymphatic network. The inset is a cartoon of the perivascular space, a fundamental component of the glymphatic system (the black box in the main figure indicates the region of interest). Astrocyte endfeet (green) connected by gap junctions (brown) and expressing aquaporin 4 (AQP4) water channels (purple) surround the perivascular space (blue) where CSF and ISF flow (blue arrow). The inside of the perivascular space is defined by smooth muscle cells (tan/gray) or by pericytes partly surrounding capillary endothelial cells (pink). (Figure by Dan Xue.)

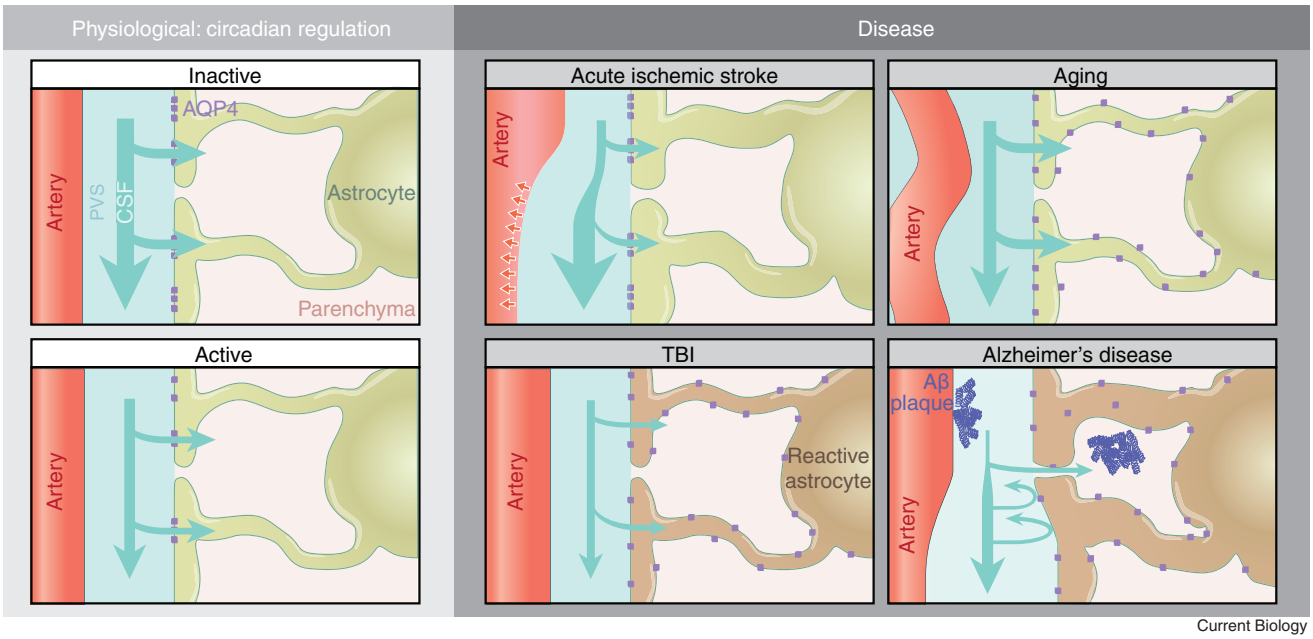

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Figure 2. Glymphatic function changes across physiology and pathology.
For all images, arteries are shown in red and the perivascular space (PVS) is in blue, with the blue arrows indicating how CSF influx changes under each condition. Astrocytes are shown either as green when healthy or as brown to indicate reactive gliosis and express the water channel AQP4 (purple squares): astrocytes are on the right of the perivascular space with the brain parenchyma shown in tan. (Left) In a healthy brain, glymphatic function changes across the circadian cycle. During the inactive phase, CSF influx is increased. The astrocytes that line the perivascular space have increased AQP4 along the perivascular endfeet. During the active phase, CSF influx is reduced and there is less perivascular AQP4. (Right) Glymphatic function can also change during the course of disease. Acute injury during stroke causes vasoconstriction and a pathological influx of CSF contributing to damaging cellular edema. Longer-term brain injury, such as traumatic brain injury (TBI), leads to increased immunoreactivity of astrocytes (now shown in brown) and mislocalization of AQP4, ultimately decreasing glymphatic influx. It is not known how normal changes in vessel tortuosity (shown as a wavy artery) in aging may lead to clinically apparent enlarged perivascular spaces in Alzheimer's disease (shown by the wider perivascular space), or how increased reactive gliosis and mislocalized AQP4 in normal aging may contribute or perhaps cause neurodegenerative diseases such as Alzheimer's disease, which present with increased arterial amyloid-β plaques (Aβ, purple blockage), and a lack of unidirectional glymphatic flow in enlarged perivascular spaces (shown by arrows of CSF flow in bottom right panel). (Figure by Dan Xue.)

humans, where magnetic resonance imaging followed by diffusion-tensor modeling of white matter have led to the suggestion that fluid movement increases in the white matter tract during sleep. Also, the clearance of radiolabeled tracer from the brain parenchyma has been shown to increase during sleep compared with wakefulness. In mice and humans the sleep–wake cycle also regulates the clearance of endogenous amyloid-β and tau proteins, which may be important in Alzheimer's disease progression. Taken together, these studies demonstrate that promoting glymphatic function might be a critical component of sleep; to clear waste and maintain brain homeostasis.

The brain has very characteristic electrical activity during sleep. Broadly speaking, sleep contains both rapid-eye movement (REM) sleep and non-REM (NREM) sleep. REM sleep is characterized by a more complex, almost awake-like brain activity, whereas NREM is characterized by synchronized, slow (1–4 Hz) brain activity that travels in a wave across the cortex. The increase of glymphatic function during sleep correlates with the prevalence of NREM. Similarly, the 1–4 Hz brain activity that is induced by anesthesia correlates with increased glymphatic CSF influx. In humans, NREM brain activity drives hemodynamic blood volume changes that increase CSF movement in the brain. Although we know that slow waves drive glymphatic flow, we do not fully understand why glymphatic function shuts down with more chaotic brain activity, such as in REM sleep or wakefulness. It is possible that the glymphatic system is 'on' during sleep to provide the essential housekeeping function of exporting metabolic waste, but also 'off' during wakefulness to avoid spillover of the excitatory neurotransmitter glutamate outside of the synapse — the active signaling area of the neuron — promoted by active fluid transport, which would compromise the precision of brain circuit function needed for higher information processing. Norepinephrine, a neurotransmitter that promotes wakefulness, inhibits glymphatic flow, supporting this hypothesis.

The two-process model of sleep states that an organism will sleep as a function of how long it has been awake and if the time of day is right to sleep. The glymphatic system shows evidence of being regulated by circadian (~24 hour) timing. In anesthetized mice, glymphatic function is highest during the inactive phase and lowest during the active phase. This rhythmicity of fluid transport is supported by similar patterns in expression of the dystrophin-associated complex and AQP4 water channels. The circadian regulation of CSF transport extends beyond the glymphatic system because

CSF exits the brain via the cervical lymphatic vasculature with the opposite pattern, that is, this flow is increased during the active phase. Although the redistribution of CSF from the brain to the lymphatic system is intriguing, how these two endogenous, circadian rhythms impact brain–body signaling remains unknown. Furthermore, whether the sleep homeostat (the 'need for sleep') directly regulates glymphatic flow is not known.

Sleep is an evolutionarily conserved phenomenon. It stands to reason that, if one purpose of sleep is to utilize the glymphatic system to clear waste, the glymphatic architecture should also be evolutionarily conserved. Indeed, perivascular spaces and evidence for glymphatic flow have been found in mice, rats, pigs, non-human primates, and humans. Even flies have an upregulated waste clearance mechanism during deep sleep, when proboscis movement drives hemolymph into the Malpighian tubules, the fly functional analog of the vertebrate kidney, to ultimately be transferred to the hindgut and secreted from the body. How the glymphatic system is organized and functions during sleep in other organisms is yet to be explored. One particularly interesting area of research would be to understand how animals such as seabirds or dolphins, which exhibit hemispheric sleep where only half the brain is dormant at a time, handle glymphatic waste clearance.

Box 1. Open questions in glymphatics research.

What is the size and shape of the perivascular space in the microvasculature? How does the structural organization of this space impact interstitial fluid movement?
What are the interstitial fluid efflux routes? Do these change based on brain region, physiology, or disease? How do these routes interface with the peripheral lymphatic system?
What are the regulatory mechanisms differentiating local (within brain region) compared with global (across the brain) glymphatic flow?
Is glymphatic flow regulated by the sleep homeostat? Does sleep restriction/disruption actually change perivascular flow?
Could 'turning off' glymphatic flow be just as important and tightly regulated as 'turning on' this flow for brain function and long-term health?
Can diminished glymphatic function trigger disease or merely accelerate disease progression?

Glymphatics and disease

The glymphatic system has been implicated in a wide variety of neurological diseases, from stroke and traumatic brain injury to Alzheimer's disease and dementia (Figure 2).

More recent analysis shows that the initial tissue swelling that occurs after stroke can be regarded as 'glymphedema' — a build-up of fluid in the glymphatic system. Loss of blood flow in the brain initiates spreading ischemia, a wave of synchronized neuronal and astrocytic depolarization that spreads across the cortex from the initial site of ischemia. Spreading ischemia triggers vasoconstriction and a widening of the perivascular space. The subsequent rapid influx of CSF along the enlarged perivascular spaces is responsible for the edema and likely contributes to the ensuing secondary injury. How glymphatic function changes in later stages of stroke, where a systemic immune response has been triggered and damaged tissue must be cleared and repaired, is yet to be tested. In models of traumatic brain injury, glymphatic function is diminished and clearance of debris is impaired for prolonged durations. It is possible that diminished glymphatic function is a hallmark of chronic brain injury, and that interventions that target glymphatic function could improve tissue healing.

Glymphatic function declines not only after brain injury, but also with natural aging. This is associated with increased immunoreactivity of glial fibrillary acidic protein in astrocytes and mislocalization of AQP4 away from the astrocyte vascular endfeet to the soma and perisynaptic processes. The loss of AQP4 vascular polarization is linked to reduced CSF influx. Additionally, tortuosity of the vasculature increases with age, potentially slowing CSF flow into the perivascular space due to the increased vessel complexity. How this decline in glymphatic function with age translates into changes in cognitive status and/or neurodegeneration is still under investigation. Enlarged perivascular spaces in the cortex are associated with increased prevalence of dementia. Mouse models of neurodegenerative diseases such as Alzheimer's have increased amyloid-β and tau proteins in the brain, and further decrease of glymphatic flow. Whether a decline in glymphatic function with aging actually triggers neurodegenerative diseases or accelerates protein deposition is still an open question in the field.

Closing remarks

The hypothesis that CSF and ISF mix and enable waste clearance through the perivascular space was proposed as early as the 1870s, and the activation of this process during sleep was proposed in the 1890s. What brought the glymphatic system to the forefront of modern neuroscience was the advent of *in vivo* microscopy to establish directionality of flow, the ability to implicate astrocytes and AQP4 water channels in glymphatic flow through our current understanding of the genetic and cellular composition of the brain, the observations that amyloid-β and tau could be cleared by this pathway, and, finally, the demonstration that sleep was essential for clearing waste from the brain in live animals. Studying this system holds a strange place in science, entrenched in hundreds of years of stellar research but hindered by old models, methodology and techniques. The structure of the glymphatic system is completely altered after death because the perivascular spaces collapse once blood perfusion ceases. Also, cell volume is altered in histological preparations, making it impossible to make predictions about dynamic fluid pathways in fixed tissue. To answer fundamental questions about this system (Box 1), whether it be understanding the role of astrocytes and blood vessels in the control of perivascular flow, what exact pathway(s) ISF uses to exit the brain tissue, or which comes first, glymphatic decline or neurodegeneration, we will need fresh perspectives, new techniques, and collaborations across disciplines, not only within neurobiology and sleep physiology, but also across immunology, lymphatic biology, fluid dynamics and more.

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Historical body temperature records as a population-level 'thermometer' of physical activity in the United States

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Over the past two centuries profound technological and social changes have reduced overall levels of physical activity (PA). However, just how much population-level PA levels have declined since the Industrial Revolution is unknown because methods for measuring PA, such as accelerometry and the doubly labeled water technique, were developed only within the last few decades. Here, we show that historical records of resting body temperature (T_B) can serve as a 'thermometer' of population-level PA, enabling us to use the well-documented secular decline in T_B in the US[1] to approximate PA decline in the US since 1820. Using cross-sectional data relating T_B to resting metabolic rate (RMR) and RMR to PA, we estimate that RMR has declined by ~6% and moderate to vigorous PA by ~27 minutes per day since 1820 in the US.

Although T_B of healthy adult humans is canonically considered to be 37°C (98.6°F), T_B varies on a circadian basis, declines with age, and has declined at the population level since the first large-scale measurements began in the 19th century[2]. In a large-scale analysis of data from the past two centuries, Protsiv *et al*.[1] showed that T_B in the US declined steadily since 1820 at a rate of 0.03°C per decade in both men and women. At a proximate level, T_B and RMR are directly related, as first demonstrated by Dubois, who reported that every 1°C increase in T_B among febrile patients was associated with a 13% increase in RMR[3]. Although Protsiv *et al.*[1] suggested that the 0.59°C decline in men's T_B since 1800 and the 0.32°C decline in women's T_B since 1890 resulted from reduced levels of inflammation, T_B decreases did not decelerate with declines in the burden of infectious disease, which have been steady since the mid-20th century[4]. Furthermore, a similar decline in T_B was observed among Bolivian horticulturalists undergoing rapid market integration (transitioning from subsistence farming to buying and selling food, labor and other goods in markets) over the last 20 years without any change in pathogen loads[5]. These lines of evidence suggest that other factors that influence RMR, among them PA, might also be responsible for the observed secular trend in T_B.

The first step to estimate population-level changes in PA from T_B is to relate T_B to RMR across individuals. We analyzed the between-individual relationship of RMR and T_B using a sample of white male participants in the Minnesota Semi-Starvation Study[6]. After controlling for body mass and body fat percentage, our model found that every 1°C increase in T_B was associated with a 10 ± 7% change in RMR (Supplemental information). Applying this conversion factor to the T_B decline from Protsiv *et al*.[1] results in an estimated RMR decline of 0.3% per decade, approximately 6% in total since 1820 (Figure 1A).

We next evaluated how much population-level declines in PA could explain our estimated declines in RMR over the same time period. Temporary elevations in RMR following PA are a well-established phenomenon termed excess post-exercise oxygen consumption (EPOC)[7]. EPOC declines rapidly within the first several minutes after cessation of PA but can persist as a low-level elevation in RMR for up to 48 hours depending on several factors including fitness level, age, sex, and PA dose, with the overall magnitude of EPOC increasing linearly with duration, and exponentially with intensity[7]. For example, 8 adults who exercised for 80 minutes at 70% VO_2max increased their average RMR by 5% over 24 hours[8].

To approximate the extent to which a reduction in PA could result in the 6% decline in RMR that we estimate occurred over the industrial era in the