CHAPTER TWO

THE HUMAN BODY AND MIND; WHAT IS PERFECT AND WHAT CAN GO WRONG

Clearly this is neither a textbook of anatomy, physiology or human biology, nor of biomechanics or biophysics. In explaining, however, what we can do to improve the human body, we must address what it is that we start with, and why we may not consider the form that we have been dealt at conception, or which has been adapted through life, to be adequate. In elementary texts there are several alternative methods to characterize the essential functions of the body (movement, respiration etc.), and somewhat fewer, and rather more vague, ways to distinguish the essential features of the mind (thinking, feeling etc.). These do not necessarily help us in the present context of defining an acceptable human structure; it is better to identify those functional attributes of ourselves that determine our performance and well-being, and which are, or could be, amenable to medical intervention. It will be demonstrated here (and this should be of no surprise) that the chronology of such interventions follows the pattern of, first, the simple practical solutions to address the most obvious deficiencies (e.g. a wooden leg for an amputee, or a glass eye or ivory tooth) right through to the almost science-fiction approaches to the fully-functional, pseudo-artificial organs that are able to restore all manner of activities – the so-called 'bionic man' in the public mind and, regrettably, now in official documents of important governmental institutions.

2.1 FUNCTIONALITY AND PERFORMANCE

The spectrum of the body's functions that can be considered as targets for, or goals of, reconstruction is represented in the following Table.

Body System	Primary Generic Function	Specific Functions	Major Tissues / Organs	Reconstructive Achievements
Musculo-skeletal	Biomechanical	Support, form, movement	Muscles, bones, tendons, cartilage	*****
Cardiovascular	Biomechanical and Biophysical	Control of blood circulation	Heart, arteries, veins, blood	****
Sensory / nervous	Biophysical	Communication, coordination, sensing	Brain, nerves, eyes, ears, skin, nose etc.	**
Urinary	Biochemical and Biomechanical	Waste elimination	Kidneys, bladder, urinary tract	**
Integumentary	Biomechanical and Biophysical	Protection, Aesthetic	Skin, hair, nails	**
Respiratory	Biomechanical and Biochemical	Breathing	Lungs, Airway organs	*
Digestive	Biomechanical and Biochemical	Processing food	Mouth, esophagus, Stomach, intestines Accessory organs	*
Hematopoietic Lymphatic	Biochemical	Blood production, Defense	Bone marrow, spleen, Lymph fluid and nodes	*
Endocrine	Biochemical	Hormone control	Thyroid, pituitary, adrenal, other glands	-
Reproductive	Biochemical	Reproduction	Uterus, ovaries, testes	-

Table 2.1. Major systems of the human body, their functions and reconstruction targets.

2.1.1 Biomechanical Considerations

The first group of functions involves the biomechanical operations of the human body. Biomechanics is concerned with the action of external and internal forces on and in the body. When the forces are structural, these actions affect all manner of movement and posture; they are critical to the operation of sensory organs, as with eye movement, and of nutrition, for example, chewing. When the forces relate to fluids, they are involved with blood circulation, respiration, nutrition (again), with salivary gland function, excretion, interstitial flow within most tissues, synovial joint function and so on. The restoration of biomechanical function is not trivial, but many early interventional devices and procedures provided some relief from the pain, discomfort and inconvenience associated with limited mobility, including the use of dentures, artificial prosthetics, orthotics and, at a later stage, internal devices to help fractures heal and a variety of medical carpentry-like appliances to correct obvious deformities such as the spinal abnormalities of scoliosis or lordosis. One of the most common conditions affecting the musculoskeletal system is osteoarthritis, quite simply the age-related destruction of cartilage and underlying bone of the synovial joints, the treatment of which by total joint replacement must rank as one of the most successful of surgical interventions introduced in the twentieth century. All the above techniques are solely based on the requirement to mechanically replicate load-bearing structures; generally, no other functions are required. There are, of course, some difficult, perhaps borderline, areas where biomechanics plays a role in perception rather than mechanical movement, such as in the sensation of touch; these will be dealt with separately alongside other aspects of mechanobiology.

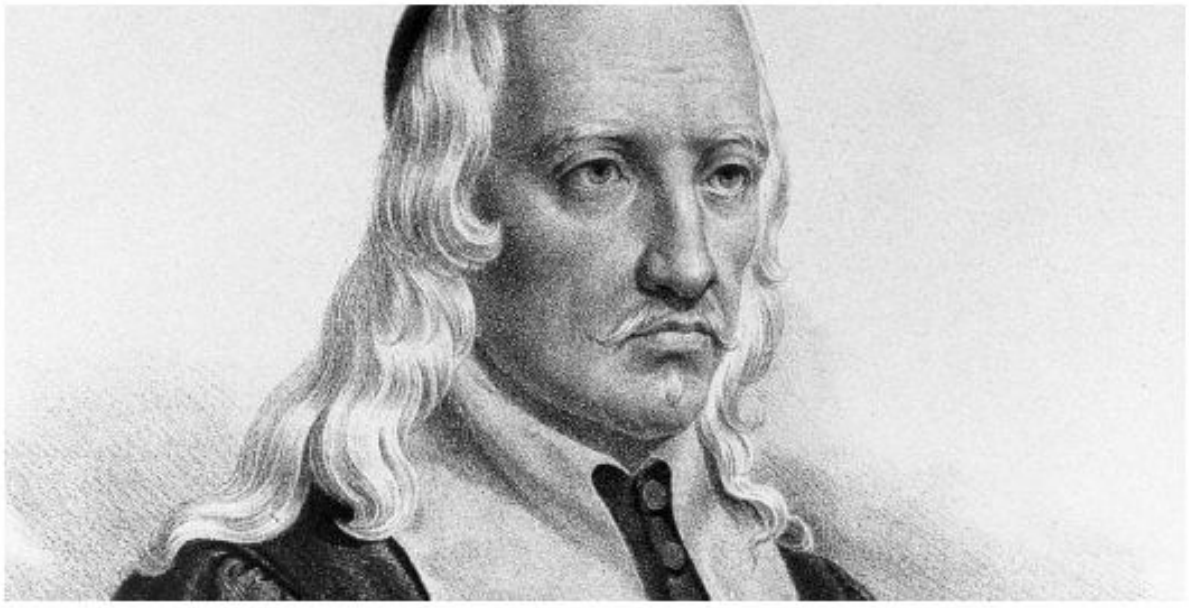

Figure 2.1. Giovanni Alfonso Borelli; founder of the science of biomechanics. He showed that the motions of animals was caused by muscle contractions and the mathematics of levers, pulleys and other machines applied to the components of the body, rejecting the view that motion was caused by a vital fluid in the muscles coming from nerves.

The restoration of the fluid mechanical environment is somewhat more difficult since the fluid channels are virtually all internal structures. Fluids interact with these channels in significant ways and control of the flow is often required through series of valves. The interaction of blood with reconstructed or replaced channels, as with artificial arteries, is a major limiting factor. Outside of the major circulatory system, the

challenges are even more significant, and very little has yet been achieved with real control of fluid movement in the eye, the inner ear, the spinal cord, or the mouth. In reality, the replacement of fluid flow function involves the passive introduction of tube-like structures into the body without any pretense of control of the flow in any meaningful physiological way. In some situations, one-way valves are used to influence directionality of the flow, and in other situations the procedures are used to block flow altogether when that flow is unnatural (as with congenital heart defects) or undesirable (some implantable contraceptive devices). As with structural biomechanical functions, the concepts are usually very simple, and no other functions are required.

2.1.2 Biophysical Considerations

Biophysics, the application of the laws of physics to biological phenomena, constitutes a somewhat higher level of complexity in the functional restoration of the body since it may involve the application of physical stimuli to override pathologically derived dysfunction, often with sensing and feedback characteristics. The physical stimuli may be auditory, electrical, optical, optoelectronic, magnetic, or electromagnetic.

Until recently, the relevant electrical functions largely referred to disturbances in the conduction systems of the heart, giving rise to arrhythmias, where a higher-than-normal heart rate, tachycardia, or lower-than -normal rate, bradycardia, can be distressing, disabling or fatal, as with, for example ventricular fibrillation, the disorganized firing of impulses from the ventricles. As we shall see, there are multiple sources of conduction error and there are many types of device that can be used to superimpose a more natural heartbeat, either continuously or on-demand. Cardiac pacemakers are most commonly used for the application of stimuli, but there has been increasing use of more complex systems, for example the implantable cardioverter defibrillator, which can detect fibrillation and correct it by application of a significant electric shock. The latter are now very sophisticated devices, with sensing and remote monitoring capability. There are two main mechanisms of arrhythmic disturbance, it being caused either by abnormal cellular activity that gives variable impulse formation or by obstacles or obstructions to conduction in the nerves. These mechanisms of abnormality may be entirely of genetic origin but are influenced by infection, physical or emotional stress, disease (including heart disease) and the use of stimulants such as recreational drugs. In many disease states, the first lines of therapy (i.e., before replacement therapies are considered) may be either life-style changes or pharmacological approaches that ameliorate these influences. The devices that are used are usually fully implantable, rather than having extracorporeal power supplied linked to internal stimulators, as they were in the early days, and their technology stretches properties such as hermeticity, insulation, biocompatibility, and fatigue resistance to their limit.

Other conditions that may be treated with devices that stimulate nerve tissue may be based on entirely different types of pathology or dysfunction. Foremost here are the conditions that require neuromodulation, that is the targeted delivery of stimuli, for example electrical signals, to specific neurological sites, where the stimulus modulates abnormal neural pathway behavior, giving relief of pain, restoration of bodily functions such as bladder and bowel control, or tremor suppression.

With the eye and the ear, the most common functional deficiencies are usually quantitative, that is whether sufficient light and sound energy can get to the points where that energy is transduced to electrical signals within the nervous system, preferably with minimal distortion. This could be achieved with ‘external devices’ such as the early spectacles or hearing aids and then with more sophisticated passive internal devices such as the transplanted cornea, intraocular lens, or middle ear implants. Modulation of the biophysical functions first came with the cochlear implant, in which an external sound processor converts auditory signals that are transmitted to the non-functioning inner ear where they are

transduced into electrical signals for transmission along the auditory nerve. In the eye, the situation is far more complex since the parameters of efficient light energy transduction at the retina are immensely more multifactorial compared to the sound transduction in the inner ear. Nevertheless, significant advances in retinal prostheses and implants, such as epiretinal implants, and stem cell therapies, in recent years are offering considerable hope in this difficult area.

Interventions within biophysical domains have other significant differences to those of purely biomechanical characteristics, since most of them impact on nervous or sensory systems, which mean that perceptions of function may be as important as the functions themselves. I remember attending a clinic at a major London hospital way back in the 1980s, soon after the cochlear implant had been made available. A child born profoundly deaf was being fitted with this type of implant. Imagine someone who has never experienced sound suddenly hearing 'noises' of which he / she had no previous conception; the staff in the clinic included not only biomedical engineers like myself, audiologists and ENT surgeons, but also a whole array of pediatricians, psychologists and speech therapists who were aware of the potential disturbances that may arise in the child. Later in this book we shall consider many examples where replacement of functions in the body impact on psychological or even metaphysical processes.

2.1.3 Biochemical Considerations

Someone must have estimated the number of different types of chemical / biochemical reactions that take place in the human body; the exact number doesn't matter here but it is likely to be in the millions and such reactions are taking place constantly in all relevant cells in that body. This gives enormous scope for error. If we define a biochemical reaction as a chemical reaction that takes place inside cells, it is easy to see how biochemical disturbances can have a powerful influence on the overall functioning of our bodies. A couple of broad considerations suggest that the magnitude of the potential problem may not be quite so overwhelming. First, there is a considerable latitude in what may be deemed acceptable or unacceptable reaction parameters. If we undertake a blood test and look carefully at our own data, and compare that data to reference ranges, we may see a huge variation in levels of individual molecules in the general population, but where no clinically relevant conditions arise with levels in that range.

Secondly, homeostatic mechanisms, the processes by which cells keep the conditions inside them essentially the same despite changes in the surrounding environment, continually adjust the biochemical reactions so that they produce an effective equilibrium. Thus, the body is able to accommodate, without any appreciable change to the quality of life, a wide range of variables in biochemical status.

In those situations where effective accommodation is not possible, the balance may be redressed to some extent by interference either with the reactions themselves or by artificially adjusting the level of biochemicals, in the body as a whole or within discrete compartments. This is most often achieved pharmacologically, that is by administering to the patient a regular dose of the deficient biochemical or of a precursor that is converted to that substance at some target point. Vitamins provide a simple example, vitamin D being provided by orally administered supplements, especially recommended for older people in sun-deprived climates. With increasing complexity, we can see that biochemical pathways may be contributory factors in both epilepsy and depression, and the pharmacological approach to these pathways has received much attention. I do not generally cover these approaches in this book since most cannot be considered as parts of reconstructive or regenerative medicine; however, because the temporal and regionally specific nature of the molecular delivery may require far more sophistication that the swallowing of a pill, I do address some of the technological approaches of advanced drug and molecular delivery.

Of more relevance are those conditions where chronic disturbances to biochemical pathways have a significant effect on the quality of life, and indeed on the existence of life itself. Two principal diseases come to mind, type I diabetes, and chronic kidney disease. The former involves the inability of the pancreas to produce insulin, which is required for the processes of glucose entry into cells where energy is produced, while the latter is associated with multiple physiological and metabolic disturbances that prevent normal kidney function, resulting in the accumulation or uremic toxins in the patient. Type I diabetes is treated by regular, lifetime, administration of insulin, either by injection or infusion pumps; the possibility of a tissue engineered pancreas has been a target for regenerative medicine that will be discussed later. There are no pharmacological therapies that can address the causes of chronic kidney disease because of the multiplicity and complexity of the involved pathways, so that the major long-term therapeutic options involve either whole-scale treatment of blood to remove the toxins in an extracorporeal machine, or total kidney transplantation.

2.1.4 Aesthetic and Metaphysical Considerations

Aesthetics is the philosophical study of beauty and taste. Metaphysics is that division of philosophy that is concerned with the fundamental nature and origin of reality. Ontology and epistemology are two forms of metaphysics, the first dealing with the structure of the nature of reality and the latter with the nature of knowledge itself.

The issue at stake here in this book, and specifically in this chapter, is whether aesthetics and metaphysics are at all relevant to the reconstruction of the human body and, consequentially, whether considerations of these factors are aligned with those discussed in the three sections above on the objective measures of functionality.

2.1.4.1 Aesthetics

It has been argued that to consider more than the general definition of aesthetics given above is very difficult, since it relates to highly subjective experiences, incorporating domains of the beautiful, the ugly and the elegant, and of taste and enjoyment. If similar principles operate, and similar interests are engaged within and between these domains, we have the basis of philosophical aesthetics, but if they do not, concepts such as beauty and taste can have only marginal philosophical relevance. Almost anything might be seen as beautiful by someone, and application of beauty to highly diverse, even discordant, objects suggests that they have little or nothing in common. On the one hand there may be some single underlying belief that motivates all judgments, but conversely the term beautiful may have no sense except as the expression of an attitude, which is in turn directed by different people to quite different situations.

Interestingly, to my mind, one of the best essays about beauty was written 200 years ago when the Right Hon. Edmund Burke, of London, published his inquiry into the origins of ideas on the sublime and beautiful[1]. It would not be relevant to discuss these ideas in detail here, but I note his inclusion of aspects of passion, feeling, pain, proportion, perfection, delicacy, elegance, power, color, darkness, blackness and variety in his analysis of the perception of beauty. In the more mundane world of the English language, beauty is usually equated with '*something that gives pleasure to one's senses'*, which doesn't really help since pleasure is equally subjective. And to add to the confusion, one of the most revered lines in poetry contains the phrase '*a terrible beauty*':

[1] Burke, Edmund, A Philosophical Inquiry into the Origins of Our Ideas on the Sublime and Beautiful, Thomas McLean, London, 1823.

We know their dream; enough
To know they dreamed and are dead;
And what if excess of love
Bewildered them till they died?
I write it out in a verse—
MacDonagh and MacBride
And Connolly and Pearse
Now and in time to be,
Wherever green is worn,
Are changed, changed utterly:
A terrible beauty is born.

William Butler Yeats, "*Easter, 1916*"[2]

Yeats was writing about The Easter Rising in his native Ireland. He conflated admiration for the rebels with the admission that the rebellion was futile. Beauty itself can cause similar conflation of contradictory feelings.

Figure 2.2. William Butler Yeats: Author of the lines *'A terrible beauty is born'.*

[2] W. B. Yeats, 'Easter, 1916', In "The Collected Poems of W B Yeats", Scribner,1996 ISBN 0684807319.

The use of beauty as a metaphor has not been confined to literature. Paul Dirac, an eminent English theoretical physicist, used the concept of beauty to describe the work of Heisenberg, Einstein, and Schrodinger[3]:

> *The big advance in the quantum theory came in 1925, with the discovery of quantum mechanics. This advance was brought about independently by two men, Heisenberg first and Schrodinger soon afterward, working from different points of view. Heisenberg worked keeping close to the experimental evidence about spectra that was being amassed at that time, and he found out how the experimental information could be fitted into a scheme that is now known as matrix mechanics. All the experimental data of spectroscopy fitted beautifully into the scheme of matrix mechanics, and this led to quite a different picture of the atomic world. Schrodinger worked from a more mathematical point of view, trying to find a beautiful theory for describing atomic events, and was helped by De Broglie's ideas of waves associated with particles. He was able to extend De Broglie's ideas and to get a very beautiful equation, known as Schrodinger's wave equation, for describing atomic processes. Schrodinger got this equation by pure thought, looking for some beautiful generalization of De Broglie's ideas, and not by keeping close to the experimental development of the subject in the way Heisenberg did.*
>
> *I think there is a moral to this story, namely that it is more important to have beauty in one's equations than to have them fit experiment.*

Since the concept of beauty has been used to reflect on the usefulness, or appropriateness, of human conflict, physicist's equations, and many other things[4], without much in the way of commonality, we may be forced to take a more utilitarian approach to its relevance to the reconstruction of the human body. At its very basic level, and using evolutionary theories, aesthetic criteria or standards for female beauty are simple signals of fertility, while the aesthetic standards for male beauty are the attributes for hunting and protection. Darwin himself worried about how the existence of 'minds' fitted into this general concept, and we have to recognize that consciousness has a role in evolution[5].

With respect to reconstructing, or perhaps re-shaping, the body, we should consider beauty in what could be described in either 'macro' (i.e., generic) or 'micro' (i.e., individual) terms. In a recent extensive study of ideas of facial beauty in South-East Asia countries where aesthetic facial surgery has become very popular[6], it was found that although there was heterogeneity with respect to some specific features and shapes that are considered beautiful, the broader ideals of beauty and of the goals of beauty improvement techniques are universal. Several studies have shown that symmetry, harmony, and balance are, globally, the key features of facial beauty and that perception of attractiveness is consistent independent of race, nationality, or age. Rather similar conclusions have been reached in relation to facial aesthetics in western cultures. Specifically with orthodontic / orthognathic procedures there is widespread, even universal, agreement as to what features constitute acceptability, and these are summarized in one word 'averageness'; beauty could be defined generically as the average values of the features of faces in a

[3] Dirac P. The evolution of the physicist's picture of nature, *Scientific American,* 1963;208:45-53.
[4] Benner SA. Aesthetics in synthesis and synthetic biology, *Current Opinions in Chemical Biology*, 2012;16:581-5. doi:10.1016/j.cbpa.2012.11.004.
[5] Smith CUM. Darwin's unsolved problem: The place of consciousness in an evolutionary world, *Journal of the History of Neuroscience;* 2010;19:105-20. doi:10.1080/096447040903504781.
[6] Samizadeh S & Wu W. Ideals of facial beauty amongst the Chinese population: Results from a large national surgery, *Aesthetic Plastic Surgery,* 2018;42:1540-50. doi:10.1080/s00266-018-1188-9.

human population[7]. The commonly quoted concept that beauty is in the eye of the beholder (anticipated in 1588 by Shakespeare in *Love's Labour's Lost* as "*Beauty is bought by judgement of the eye*") plays only a peripheral part since most patients and their treating doctors want corrective therapies that make them look normal, perhaps with just a hint of cuteness and sexual attractiveness, which are individual features superimposed on averageness.

Also, at the micro level it must be recognized that in some cultures, very obvious shape-changing procedures are undertaken, nearly always on females, with the apparent intent of increasing attractiveness. The binding of feet in China is perhaps the most widely quoted example[8]. This practice, which is now banned, goes back several millennia. Perversely there have been claims that the forceful, painful, restricting, binding of feet makes a young girl more attractive to encourage good marriage, whilst other claims suggest that this practice put off unwelcome suitors; the concept of beauty was clearly abused here, and the process was more likely associated with the enhancement of male dominance. That was probably also behind the neck-lengthening practice in some African and South-East Asia tribes. Similar arguments have been used for the purpose of the rings; it is still practiced in countries such as Myanmar, and the women are genuinely considered to be beautiful and elegant.

Figure 2.3. Rings used to stretch necks and improve aesthetic appeal of young Asian women.

[7] Edler RJ. Background considerations for facial aesthetics, *Journal of Orthodontics*, 2001;28(2);159-68. doi:10.1093/ortho/28.2.159.
[8] Miltner LJ. Bound feet in China, *Journal of Bone & Joint Surgery*, 1937;19(2):314-9.

2.1.4.2 Metaphysics, Ontology and Epistemology

A few years ago, in a Hastings Center Report[9], Dugdale combined the writings of Aristotle[10] and of the philosopher, physician, ethicist Jeffrey Bishop[11] into a commentary on the metaphysical aspects of life, and especially the end of life. Aristotle articulated an extensive account of causation. He attempted to bridge the apparent gap between mind and matter, and to have knowledge of an object, by defining four 'causes';

- **Material Cause** - that of which the object is made,
- **Formal Cause** - the defining characteristics of the object,
- **Efficient Cause** - the antecedent condition that brought the object about,
- **Final Cause** - the purpose of the object.

He used the example of a bronze horse, where the material cause is bronze, the formal cause is the shape of the horse statue, the efficient cause is the knowledge of bronze casting, and the final cause is the production of art.

Bishop worries that in medicine today, the formal and final causes have been downplayed, with more emphasis on material and efficient causes. This questions what exactly life is; he contends that medicine has created a body in perpetual motion, re-defining human life as human function, where doctors simply replace the broken or dead parts of the human machine with other machines, so the body stays in motion. In the highly machine-driven clinical interventions, the metaphysics of efficient causation is not concerned with the flourishing of the patient: "*the patient vanishes in every sense except as an object of technological monitoring and mechanical intervention…patients in the ICU often end up living lives perceived to be worse than death, with no hope of returning to a state of human thriving*".

The implications of this position from ethical and metaphysical aspects of significant life-impacting reconstructive techniques are obvious.

So too are some of the considerations of metaphysical theories of personal identity[12]. At an elementary level, it would seem common sense that there is continuity of personal identity throughout life – an infant becomes a child, that becomes an adolescent, then an adult, then an old adult, but remains the same person over this time. But, as current philosophical discussions demonstrate, there can be many different views about the essence of this continuity, that is, what property guarantees that the person stays the same. This again reflects the metaphysical position concerning reconstruction of the body; how far can we go in reconstruction before challenging the doctrine of personal identity continuity.

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[9] Dugdale LS, Medicine's Metaphysics, *Hastings Center Report*, March – April 2013, 43(2), 7-8. doi:10.1002/hast.150.

[10] Cohen SM and Reeve CDC, "Aristotle's Metaphysics", *The Stanford Encyclopedia of Philosophy* (Winter 2020 Edition), Edward N. Zalta (ed.). https://plato. stanford.edu/archives/win2016/entries/aristotle-metaphysics/

[11] Bishop J, The Anticipatory Corpse: Medicine, Power and the Care of the Dying, Notre Dame University Press, Indiana, 2011. ISBN 9780268022273

[12] Andrade G, Clinical cases and metaphysical theories of personal identity. *Medicine, Health Care and Philosophy.* 2019;22:317-26. doi:10.1007/s11019-018-9869-3.

Generally, in this discussion, three options are provided: the soul theory, the body theory, and the psychology theory. Concepts of the soul theory, famously espoused by Descartes[13], imply that although it is an immaterial non-apprehensible substance, the soul interacts with, and indeed controls, the body; Descartes considered that these interactions took place within the pineal gland. Suffice it to say that although modern philosophers do not generally deny the existence of soul, since there is no way to visually represent it, their position is that how can it be said that it warrants the continuation of personal identity regardless of physical or mental characteristics.

The body theory, unsurprisingly, determines that an individual continues to be the same if it conserves the same body. There can be arguments about 'conservation of the body' when metabolic turnover determines that the atomic and molecular constitution continuously changes, and normal ageing processes results in changes to body features (loss of muscle tone, addition of adipose tissue) and facial features such as wrinkles and loss of hair, but it would be counter-intuitive to correlate these with lack of personal continuity. It is a little like recognition of the often-quoted position that *"I can't define beauty (or was it pornography?) but I sure know it when I see it".* However, we should be more cautious when we consider the metaphysical effect of major reconstruction of the body, when a substantial part of the body is now synthetic or transplanted from another person (or possibly animal). Organ transplantation may reflect alienation within three layers of selfhood, that is embodied selfhood, self-reflection, and social-narrative identity[14]. First, a bodily alienation may be experienced during illness or injury and recovery. Where the organ is considered to harbor the identity of a different person, due to its symbolic or expressive qualities (the heart and face), the alienation process may also another person's identity. These aspects will be discussed in sections dealing with these transplantation procedures.

The third possibility is psychological theory, which centers on the brain rather than the whole body, since the brain is the focus of mental life; however, this is itself problematic as it is not the brain *per se* that relates to personal identity, but rather the psychological content. This now strays into arrays of thought experiments and analysis of very rare medical conditions aligned with so-called 'split-brains' and 'split personalities'[15]; these are outside the scope of this book, as interesting as they are.

2.1.5 Integrated Systems

It is self-evident that the systems mentioned above integrate within the human body as a whole; without such integration we would not have any meaningful life. During the last several decades, much has been learnt about the mechanisms of functional integration, for example the roles of biochemical functionality in bioelectrical phenomena and of mechanotransduction on cell behavior. Yet it has to be said that in most reconstruction therapies using conventional techniques and devices, such knowledge is not extensively utilized since the outcome parameters by which procedures are judged primarily relate to alleviating the obvious injuries and / or symptoms.

However, as we move towards far more complex goals in reconstruction, especially using the principles of personalized medicine that will be discussed later, it is imperative that we fully embrace the reality of integrated systems, especially those which involve the metaphysical as well as the physical aspects. By way of example, we can consider first the perceptions associated with reconstruction in the context of historical but naïve replacement of 'body parts', then the science fictional representation of full body

[13] Fowler CF, *Descartes on the Human Soul: Philosophy and the Demands of Christian Doctrine.* International Archives of the History of Ideas, 160, Dordrecht: Kluwer, 1999. ISBN 978-0792354734.
[14] Svenaeus F, Organ transplantation and personal identity: how does loss and change of organs affect the self? *Journal of Medicine and Philosophy*, 2012;37(2):139-58. doi:10.1095/jmp/jhs011.
[15] Gazzaniga M, Tales from both sides of the brain: A life in neuroscience, Harper-Collins, New York, 2016. ISBN 9780062228802.

enhancement, and thirdly the reality of early twentieth century progress in functional recovery. Figure 2.4 illustrates these points with, first, barely functional and certainly not integrated prostheses, secondly the hypothetically reconstructed 'six-million-dollar man' and, thirdly, very well integrated functional prostheses for extensively traumatized individuals.

Here, I take some examples of reconstructive procedures, both with and without associated hardware, to see the significance, listed in increasing order, of integration.

- An injury to the forearm, including the fracture of the radius, results in poor healing. Some titanium plates are used to reconstruct a small section of the bony structure. No additional procedures, such as skin grafts are required, and healing is now straightforward. The plates need to be biomechanically functional but otherwise there is little concern over integration into the body.

- The crown of a prominent incisor is broken. CAD / CAM techniques are used to produce a ceramic crown, which is placed on the root of the tooth with cement. From a mechanical point of view, functionally this should work very well. However, as elementary dental teaching will highlight, if the crown does not adequately fit into the surrounding dentition, occlusion, that is the relationship between the maxillary and mandibular teeth, is disturbed, potentially leading to migraines or temporomandibular joint dysfunction. In addition, the dentist will go to great lengths to match the color of the artificial crown to those adjacent. Clearly, biomechanical and aesthetic integration are very important for a satisfactory outcome.

- An osteoarthritic hip eventually needs to be replaced; several types of joint replacement are available and perform extremely well in most patients, with good integration between the device and the surrounding bone. Clinical technique is of prime importance, however, including attention to detail with respect to orientation and leg length. Poor anatomical integration is likely to result in poor gait, which itself can lead to greater stress on other joints, especially the knees and contralateral hip, potentially accelerating their arthritic demise, and diminution of self-esteem as the difficulty in walking makes the patient look a lot older.

- A middle-aged woman diagnosed with breast cancer undergoes unilateral mastectomy, with replacement of lost tissue with a silicone implant. Poor sizing and incorrect placement can really spoil the outcome. Although breast implants are often considered to be primarily of cosmetic value, in mastectomy patients it is restoration of normality that is paramount. Inadequate integration into the 'natural' shape, can have powerful consequences.

- A burn wound has affected a reasonably large area of skin in a prominent position. Fill thickness skin grafts are required to produce a regenerated area of skin but here is an inadequate supply of skin from other parts of the patient's body and so cadaveric skin grafts are used. It is very difficult to match the characteristics of the graft (that is pigmentation, elasticity, hair density etc.). While good skin functionality, which includes resistance to infection, may be perfectly acceptable, that area of skin may never be aesthetically integrated; this may not be too important for some people, but there are many who are psychologically disturbed by this situation.

- Patients with cardiac arrythmias may be fitted with an implantable defibrillator. This is likely to be a life-saving therapy, but full integration of the complex biophysical construction of the device with the even greater complexity of the natural conduction system is not trivial. Patients may therefore experience false negatives, when the device does not deliver the required shock, or false

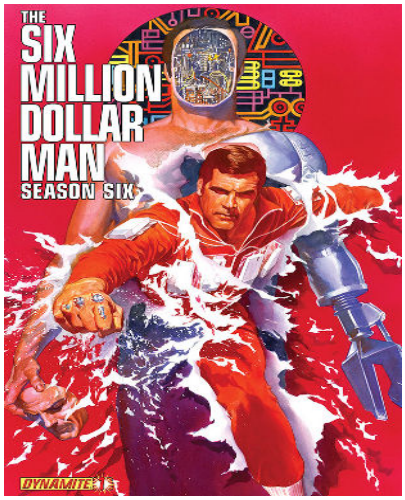

Figure 2.4. Top; barely functional and certainly not integrated prostheses. Center; the hypothetically reconstructed 'six-million-dollar man'. Lower, very well integrated functional prostheses for extensively traumatized individuals.

positives, when the patient unexpectedly, and traumatically, receives a shock when it is not required, representing a significant confluence of biophysical and psychological considerations.

This list is long enough to make the point, and we haven't even discussed the huge integration challenges with major transplantation procedures, up to and including face transplantation.

2.2 THE ANATOMICAL HIERARCHY OF THE BODY RELATED TO FUNCTION

In the light of these functional characteristics of the natural human body and their translation to the reconstructed body, it is necessary to assess the hierarchical arrangement of structural elements of which it is composed. This is achieved through a top-down approach, although this should not be interpreted as an order of importance. Each section is discussed in terms of the 'perfect' condition and the main reasons why these components become dysfunctional.

2.2.1 Organs

2.2.1.1 Brain

It is tempting to assume that the brain is so complex that it could never be the subject of reconstruction, or even transplantation / replacement, such that it could be excluded from this discussion. However, there are some extremely important aspects of brain anatomy and function that are potential targets of reconstruction, and we should not ignore the impact of artificial intelligence on future performance of the body. The section will be relatively brief; I deal with other parts of the central nervous system in a following section, although we must appreciate the integrative aspects of all nervous system components.

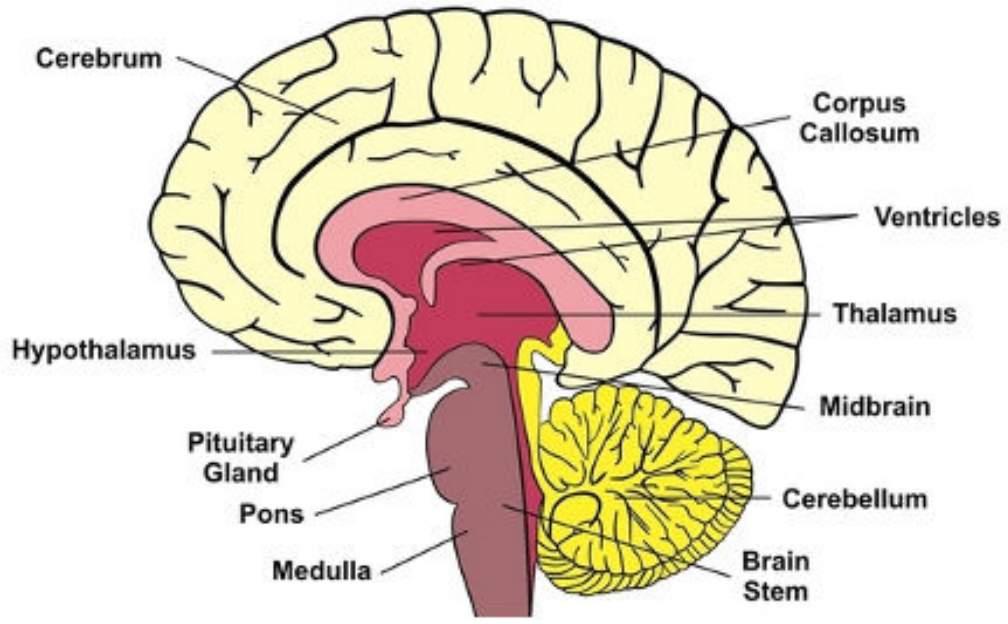

Figure 2.5. Structure of the human brain.

At the macrostructure scale, the brain is contained within the cranium, the bony structure that provides protection of the brain from injury, and which, together with the facial bones, constitutes the skull. The brain itself is separated from the skull by three layers of soft tissue, which provide a further degree of protection from mechanical forces. These layers are the dura mater (on the outside), the arachnoid and, on the inner side, the pia mater. Collectively, these three layers comprise the meninges; they have varying elasticities and vascularity and contain several fluid-filled spaces, including the sub-arachnoid space. The fluid within these spaces is the cerebrospinal fluid (CSF), which is produced by specialized structures, the choroid plexus, that are found within hollow channels in the brain called ventricles. The CSF circulates in the brain and around the spinal column. In some congenital defects, especially associated with spina bifida[16], and also following some cases of traumatic brain injury[17], the passage of CSF from the ventricles to the spine is obstructed, giving the condition of hydrocephalus, which can be resolved by surgical reconstruction, with or without the assistance of plastic shunts. The meninges are particularly prone to inflammation and viral infections.

The brain itself consists of the brainstem, the cerebellum, and the cerebrum, together with a variety of secondary systems. The brainstem sits at the base of the brain and connects with the spinal cord. It is the control center for many involuntary vital functions such as breathing and vasomotor control, and many cranial nerves are located here. It has three components, the midbrain, the pons and medulla oblongata. Disorders, and loss of certain brainstem functions, caused by trauma, infection, tumors, or strokes have particularly profound effects and complete loss of function is essentially equivalent to brain death, an important factor in the discussions of donor eligibility for organ transplantation. The cerebrum comprises the major part of the brain, consisting of two hemispheres, the right and the left, which are joined at the base by the corpus callosum. The cerebral cortex is grayish brown, hence the name ‘gray matter’ and contains a variety of grooves and fissures. Each hemisphere has a frontal, temporal, parietal, and occipital lobe. The frontal lobes are the largest and control motor skills of voluntary movement, speech intellectual and behavioral functions; the prefrontal cortex is important in memory, intelligence and concentration and the premotor cortex guides head and neck movements and the sense of orientation. Occipital lobes, located at the back of the brain, receive and process visual information while parietal lobes interpret, simultaneously, signals received from many parts, including hearing, motor, sensory and memory. Temporal lobes are involved in visual memory and recognition, in understanding language and interpretations of emotion. The pituitary gland, attached to the base of the brain behind the nose, controls the secretion of hormones, thus playing a major role in growth and development, the function of many of the organs discussed below and the function of other glands. The thalamus relays most of the information that enters and leaves the cortex, playing roles in pain sensation, attention, and alertness.

At the cellular level, there are two types of cell, the neurons and glial cells. The neurons are responsible for the transmission of nerve impulses. Glial cells, on the other hand, long-thought to simply provide the essential ‘glue’ for neurons, play critical roles in the functional support of the neurons. There are several types of glial cell, the microglia, astrocytes, and oligodendrocytes. Microglia effectively function as brain’s ‘immunological cells’ with important roles in inflammation and neurodegeneration. Oligodendrocytes surround and insulate neurons in order to confine and accelerate impulse transmission, while astrocytes control fluid flow in the brain, reshape synaptic connections and recycle neurotransmitter molecules.

[16] Norkett W, McLone DG and Bowman R, Current management strategies of hydrocephalus in the child with open spina bifida. *Topics in Spinal Cord Injury Rehabilitation.* 2016;22(4):241-6. doi:10.1310/sci2204-241.
[17] Yoon JE, Lee CY, Sin EG, *et al,* Clinical feature and outcomes of secondary hydrocephalus caused by head trauma. *Korean Journal of Neurotrauma*. 2016;14(2):86-92. doi:10.13004/kjnt.2018.14.2.86.

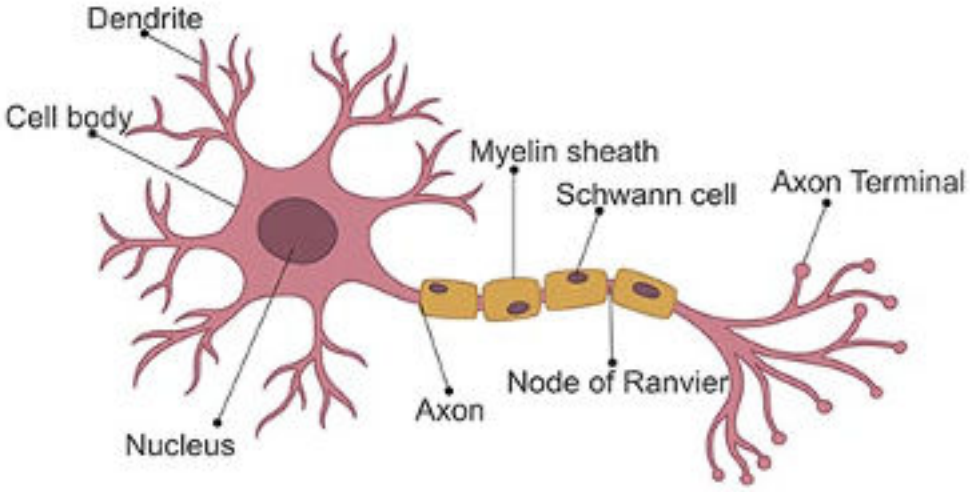

Figure 2.6. Structure of a neuron.

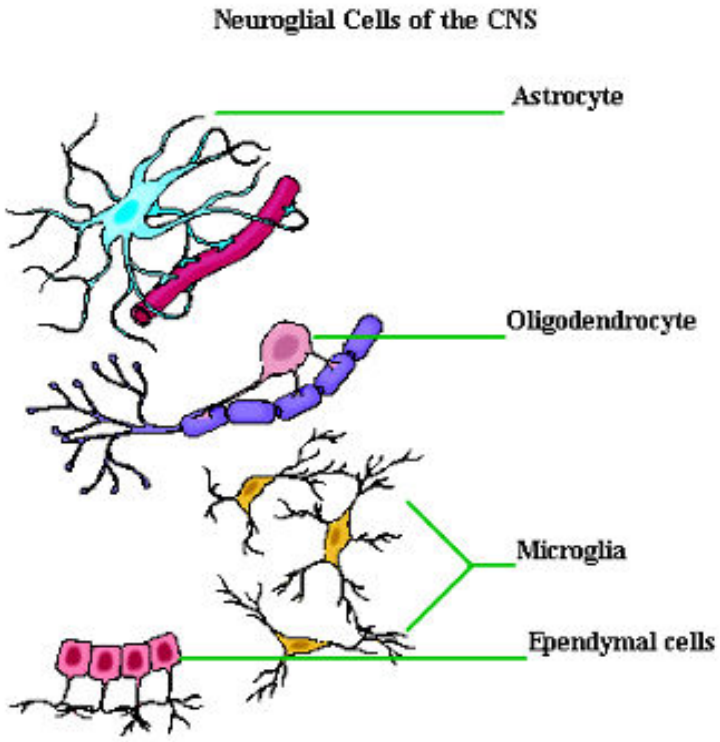

Figure 2.7. Glial cells.

It is not surprising that an organ of such exquisite, integrated, structure and performance should be susceptible to dysfunction. The major contributors here include the following.

2.2.1.1.1 Trauma

The causes and effects of trauma on the brain cover a wide spectrum. At one end are the so-called incidences of mild traumatic brain injuries, especially involving concussion, a temporary disturbance in brain function, sometimes with unconsciousness and confusion. Included here, and gaining increased attention, are those incidences of contact-sport related repetitive injuries that can result in chronic traumatic encephalopathy, a syndrome that is associated with neurodegeneration and behavioral, cognitive, and motor deficits[18]. At the other end of the spectrum are serious traumatic brain injuries, often with permanent brain damage, mental impairment, and personality changes.

2.2.1.1.2 Seizures

A seizure is a burst of uncontrolled electrical activity between brain cells that causes temporary abnormalities in muscle tone or movements and behavior. A seizure can have an acute cause, such as medication. Epilepsy occurs when a person has recurring seizures. Focal seizures can start in one part of the brain and spread to other areas, causing symptoms that are mild or severe, depending on how much of the brain becomes involved. Initially, there may be just minor symptoms, referred to as an aura. As it spreads across the brain, more symptoms appear, with feelings of confusion, muscle stiffening and fumbling. Focal seizures can spread to the entire brain and cause tonic-clonic seizures, which can cause respiratory problems and injuries. Generalized-onset seizures are surges of abnormal nerve discharges throughout the cortex of the brain more or less at the same time, usually arising from imbalances in inhibitory excitatory circuits of electrical activity. Some patients with multiple brain injuries and intellectual disability, have tonic seizures consisting of sudden stiffness in the body, which can cause falls and injuries; this is Lennox Gastaut syndrome.

As noted above, epilepsy is a major form of seizures; this affects around 50 million people worldwide; nearly 80% of those with epilepsy live in low- and middle-income countries. It is defined as having two or more unprovoked seizures. It is one of the world's oldest recognized conditions, with records dating back to 4000 BCE[19]. An Akkadian tablet dating to that period, in Mesopotamia, refers to an epileptic as having "*his neck turning left, hands and feet are tense, his wide open, and from his mouth froth is flowing without him having any consciousness*". Over several millennia, in Babylonian and Egyptian times, epilepsy was considered as the result of evil spirits, and spiritual methods were common in treatments. Hippocrates was among the first to attribute epilepsy to mechanisms within the brain and suggested that it was hereditary rather than contagious. Aristotle, on the other hand, considered that sleep and epilepsy had the same origins in relation to 'evaporations' within the vascular system. Up to the time when the electrical nature of brain function was demonstrated, in the nineteenth century, various theories about causes of epilepsy circulated, most involving evil spirits, where exorcism and other remedies were popularly applied. Even to the middle of the twentieth century, epilepsy invoked fear in observers due to its suddenness and dramatic onset, this occurring as much in advanced countries as well as those far less advanced. In the USA, people with epilepsy were forbidden to marry in many states until 1956. At that time,18 states provided for the sterilization, on eugenic grounds, of people with epilepsy. Until the 1970s, it was legal in the USA to deny persons with seizures entry to restaurants, theatres, recreational and other public places.

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[18] Pan J, Connolly ID, Dangelmajer S, *et al*, Sports-related brain injuries: connecting pathology to diagnosis. *Neurosurgery Focus.* 2018;40(4):E14. doi:10.317t/2016.1FOCUS15607.

[19] Kaculini CM, Tate-Looney AJ and Seifi A, The history of epilepsy: From ancient mystery to modern misconception, *Cureus,* 2021;13(3):e13953. doi:10.7759/cureus.13953.

The question of spirituality and epilepsy is not a trivial matter[20]. Seizure frequency and, especially, the unpredictability, are serious aspects of the quality of life of the individuals, and their families. There are quantifiable effects on distress and mental health, self-esteem, life fulfilment and several other features. There have been many studies on interactions between epilepsy and religious experiences, the use of spiritual healing in treatment and connections with mystical states, general personality and personal destiny, but with little common ground among studies. Some methods for epilepsy treatment are covered later in the book.

2.2.1.1.3 Infections

These include meningitis (alluded to above), encephalitis (usually of viral origin), and brain abscesses. These conditions are not discussed here since infections in general are discussed elsewhere in the book, and since they rarely involve reconstructive procedures.

2.2.1.1.4 Tumors

Several types of tumor are possible in the brain, one of the most serious, and essentially incurable, being glioblastoma. Glioblastomas are the most common malignant tumors of the brain and central nervous system, accounting for close to 50% of all cases, with an incidence of 3.21 per 100,000 population in the USA. Survival is poor with about 40% survival in the first year post diagnosis and 17% in the second year. It is more common in men. It is a fast-growing and aggressive brain tumor. It can arise in the brain *de novo* or develop from a lower-grade astrocytoma. Glioblastomas usually occurs in the cerebral hemisphere, especially in the frontal and temporal lobes. They can result in death in six months or less, if untreated. Factors associated with glioblastoma risk are prior therapeutic radiation, decreased susceptibility to allergy and impaired immune response. Treatment usually involves surgery, radiation and chemotherapy. The glioblastomas are surrounded by migrating, infiltrating tumor cells that invade surrounding tissues, so that removing the tumor entirely is very difficult. As with many tumors, there is no point in trying to reconstruct the area occupied prior to surgery, but reconstruction of the surrounding tissue or organ is often required, Large neurosurgical resections often provide serious challenges for surgeons, particularly with the risk of life-threatening complications, including dural exposure, cerebrospinal fluid leakage, meningitis and pneumocephalus[21]. Extensive use is often made of free-flap reconstruction of the scalp and calvaria, as discussed elsewhere[22].

2.2.1.1.5 Vascular conditions

These primarily include stroke, where blood flow and oxygenation are suddenly interrupted, causing vision, speech, sensation, and movement disturbances, depending on the area of the brain affected. Ischemic stroke may follow the development of a blood clot in an artery somewhere in the body which travels to the brain and lodges in a vessel there. Hemorrhagic stroke involves impaired blood flow caused by bleeding in the brain. On some occasions the interruption of blood flow is only temporary, giving a transient ischemic attack (TIA), where symptoms resolve quite rapidly. It is also possible for an artery in the brain to suffer an aneurysm.

2.2.1.1.6 Neurodegenerative conditions

These include Parkinson's disease, which involves a loss of neurons in an area of the brain, the substantia nigra pars compacta, and the presence of certain proteins, the alpha-synuclein, and Lewy bodies within these neurons. The substantia nigra neurons produce dopamine, a neurotransmitter that transmits signals

[20] Giovagnoli AR, Meneses RF and da Silva AM, The contribution of spirituality to quality of life in focal epilepsy, *Epilepsy and Behavio*r, 2005;9:135-9. doi:10.1016/j.yebeh.2006.04.002.

[21] Hanasono MM, Reconstruction after open surgery for skull-base malignancies, *Journal of Neuro-Oncology,* 2020;150:469-75. doi:10.1007/s11060-019-03370-1.

[22] Ray A-C, Philandrianos C, Bertrand B, *et al,* Two-stage free flap reconstruction of the scalp and calvaria for large neurosurgical resections, *Microsurgery*, 2020;40:331-6. doi:10.1002/micr.30538.

to other parts of the brain, the basal ganglia. When the neurons in the substantia nigra are affected by Parkinson's (which is either genetically or environmentally related), the loss of dopamine prevents normal function in basal ganglia and causes the characteristics of motor symptoms, tremor, and loss of spontaneous movement. In amyotrophic lateral sclerosis, ALS, or Lou Gehrig's disease, nerves that control muscle function are progressively destroyed, leading to paralysis and impaired breathing. Dementia, in general, is a decline in cognitive function associated with death and/or malfunction of brain cells. Alzheimer's disease is the most common form of dementia; it is cells within the hippocampus that are primarily affected, so that they early signs relate to loss of short-term memory and difficulties with wide-ranging social interactions.

2.2.1.1.7 Mental illness

It is assumed in this book that the range of spirituality phenomena under discussion are experienced by individuals that society consider as 'normal' and who are deemed to be 'healthy'. This is important since such phenomena must be distinguished from those experienced and displayed by those who suffer from a mental illness, who may not be considered healthy. Mental illnesses are conditions that adversely affect a person's thinking, feelings, moods, and behavior. In any year, 1 adult person out of 20 in the USA experience serious mental illness and often this remains as a permanent condition.

Among the most serious conditions here are bipolar disorder, depression, anxiety, eating disorders, obsessive-compulsive disorder, post-traumatic stress disorder and schizophrenia. Since mental illness originate in the brain, which, as we have seen, consists of just a few types of brain cell that are present throughout the various brain compartments, it would seem that such distributed conditions, whilst potentially addressable by pharmaceutical agents, would not be amenable to any form of reconstruction, regeneration or replacement therapy. However, that may not be the case. Recently, Goodkind *et al* have shown that gray matter loss in patients with those conditions listed in this paragraph converged in three regions, the dorsal anterior cingulate, the right insula and left insula, and that this lower gray matter level was associated with poor executive functioning[23]. In addition, there were some sub-regional differences, especially related to schizophrenia. Although far from suggesting that physical (e.g., surgical) interventions could target this type of localization of the origins of mental illness, it is leading towards non-invasive brain stimulation as a therapeutic modality.

2.2.1.1.8 Possibilities for brain reconstruction

Reconstruction of the brain is still largely a science fiction fantasy, but we should not dismiss the concept out of hand. Leaving aside reconstruction of the cranium and skull, which does not directly concern the brain itself, and will be considered under the general heading of craniofacial reconstruction, the only interventions in common use today can barely be described as 'reconstructive'. Microsurgery in the treatment of brain aneurysms comes close, with arterial bypass techniques[24], but these address conditions of the intracranial vasculature and not brain cells themselves. Similarly, grafts for dural reconstruction and the prevention of CSF leaks, for example in treatment of tumors of the meninges[25], do not relate directly to brain cells and their function.

Most procedures that are invasive of the brain itself are aimed at destructive therapies for curing brain conditions, or alteration of the biophysical function within the brain. In the former category are all the procedure aimed at excising brain tumors. It should be noted that, as discussed later in this book, survival

[23] Goodkind M, Eickhoff SB, Oathes DJ, *et al*, Identification of a common neurobiological substrate for mental illness. *JAMA Psychiatry*. 2015;72(4):305-15. doi:10.1001/jamapsychiatry,2014.2206.

[24] Da Silva HB, Messina-Lopez M and Sekhar LN. Bypasses and reconstruction for complex brain aneurysms, *Methodist Debakey Cardiovascular Journal.* 2014;10(4):224-33. doi:10.14797/mdcj-10-4-224.

[25] Velnar T and Gradisnik L. Soft tissue grafts for dural reconstruction after meningioma surgery, *Bosnian Journal of Basic Medical Science,* 2019;19(3):297-303. doi:10.17305/bjbms.2019.3949.

for many types of malignant primary brain tumors has not improved much in recent years[26], but also that the sole ambition is to eradicate the tumor and not to attempt any form of reconstruction. With respect to biophysical function, this is mostly concerned with deep brain stimulation[27], which addresses symptoms but not reconstruction.

In the rather obtuse area of psychosurgery, the ill-defined interface between neurosurgery and psychiatry, there have been attempts to physically intervene within the brain in order to re-shape human behavior. These attempts have not achieved much success, the most notorious examples being seen with lobotomy[28]; popularized 75 years ago but subsequently abandoned, this involved sectioning of the prefrontal lobe and leucotomy, the severance of the underlying white matter.

Within the context of regenerative medicine, it is clear that the brain is not just a mass of cells but a complex set of circuits that have intricate spatial organization; this has led to the received wisdom that in the adult brain no functional regrowth of neurons and synaptic contacts can occur[29]. While evidence does suggest that the potential for cell renewal exists, regenerative processes do not occur to an extent that allows functional restoration. Although progress is being made with neural stem cell science, the situation today still seems to be that reflected by Arzate and Covarrubias[30] who conclude that" it *seems very challenging to generate specific new neurons de novo that have the capacity to integrate correctly into the preexisting and complex neural networks of the adult brain*".

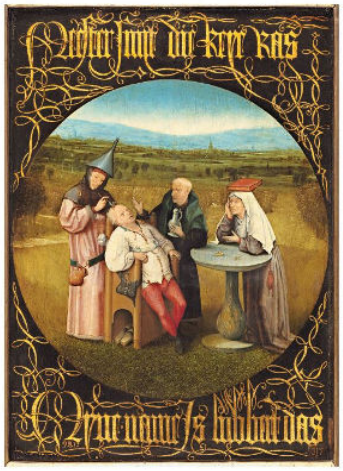

Figure 2.8. *The Extraction of the Stone of Madness* by Hieronymus Bosch – considered as anticipating neurological procedures such as lobotomy.

[26] Editorial, Focusing on brain tumors and brain metastasis. *Nature Reviews Cancer.* 2020;20:1. doi:10.1038/s41568-019-0232-7.
[27] Krauss JK, Lipsman N, Aziz T, *et al.* Technology of deep brain stimulation: Current status and future directions. *Nature Reviews Neurology.* 2021;17**:** 75–87. doi:10.1038/s41582-020-00426-z.
[28] Faria MA. Violence, mental illness and the brain; Part 1 – From trephination to lobotomy, *Surgical Neurology International.* 2013;4:49. doi:10.4103/2152-7806.110146.
[29] Martino G, Pluchino S, Bonfanto L, *et al,* Brain regeneration in physiology and pathology: the immune signature driving therapeutic plasticity of neural stem cells. *Physiological Review.* 2011;91(4):1281-304. doi:10.1152/physrev.00032.2010.
[30] Arzate DM and Covarrubias L Adult neurogenesis in the context of brain repair and functional relevance. *Stem Cells Development.* 2020;29(9):544-54. doi:10.1089/scd.2019.0208.

2.2.1.2 Heart

The heart is, obviously, very different to the brain, but consider their dialogue:

Brain: I resonate to millions of instructions every minute
Heart: I perform an exquisite function sixty times a minute
Brain: I see, hear, feel, taste, smell and think all the time
Heart: I do not let these senses affect me, I carry on regardless
Brain: Keep sending me blood, food for thought
Heart: Keep sending me rhythm, for pulsating action

David Williams, *The Heart of the Brain and the Brain of the Heart*, Unpublished poem, 2020.

Integration of heart and mind is a powerful concept, used by many artists and poets through the ages. In this section the anatomy and function of the heart are considered, along with mechanisms for so-called heart-conditions.

The heart is a predominantly muscular organ that is divided into four chambers. The two upper chambers are the left and right atria, and the two lower chambers are the left and right ventricles. Left and right sides are separated by a significant wall of tissue, the septum, while valves control flow of blood between left side chambers (the mitral valve) and between right side chambers (the tricuspid valve). Two valves also control flow of blood into and out of the heart, specifically the pulmonary valve controls flow into the right ventricle from the pulmonary artery and the aortic valve controls flow out of the left ventricle to the aorta. The phase of the heart's cycle when contraction forces blood into the arteries is known as systole, while blood re-enters the heart as it relaxes during diastole.

The heart wall essentially consists of three layers. The bulk of the muscular tissue in the central layer is the myocardium. The endocardium is the inner, blood-contacting layer which incorporates the sub-endocardium which contains the impulse-conducting system. The pericardium is the protective outer layer, which itself has two components, a thin inner epicardium, and some connective tissue. It is not entirely uncommon for that part of the heart wall that separates left and right sides (the septum) to have some form of deformity, leading to abnormal blood flow between atria and between ventricles.

The electrical conducting system involves specialized cardiac cells which distribute impulses throughout the heart. These impulses initiate at the sinoatrial node, which is located at the junction of the superior vena cava and right atrium and travel throughout the atria until reaching the atrioventricular node between the interatrial and interventricular septum. Pulses penetrate the central fibrous body of the cardiac skeleton to form the bundle of His. These fibers, (the Purkinje fibers) divide within the interventricular septum and branch into the ventricles.

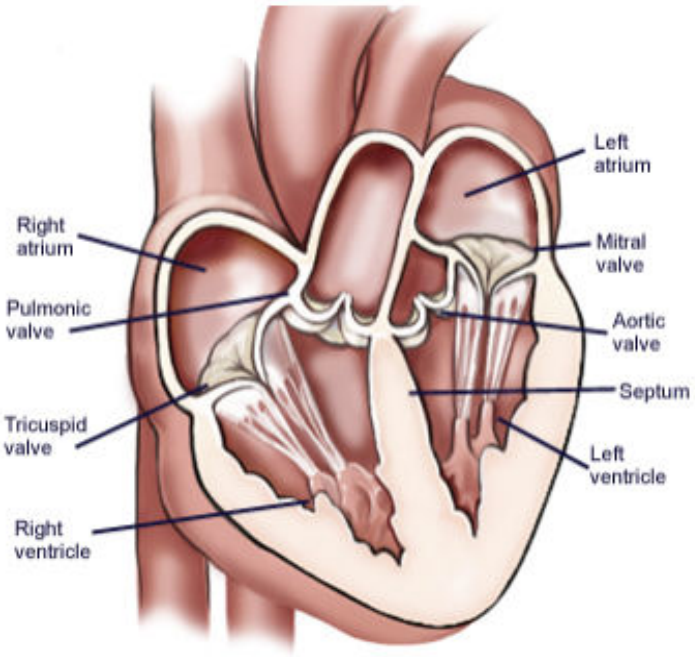

Figure 2.9. The chambers and valves of the human heart.

The valves critically control the flow of blood, both providing forward flow and preventing back-flow. Those valves that control flow into and out of the heart are semilunar valves and the atrioventricular valves have attachments to the ventricles via chordae tendinae that are inserted onto the papillary muscle. As we shall see, these valves operate under quite different hemodynamic conditions, those on the left side experiencing significantly higher pressures. In the highly stressed aortic valve, the pliable cusps have three distinct layers of extracellular matrix, which are rich in collagens, proteoglycans, and elastin, with valvular interstitial cells in their interior and valvular endothelial cells on the leaflet surfaces[31]. A great deal can go wrong during the average of 3 billion opening – closing cycles expected of them during life.

The nature and distribution of cells in the heart wall is important. The predominant cells are the cardiomyocytes, fibroblasts, endothelial cells and perivascular cells[32]. The cardiomyocytes constitute 30-40% of the cells, but because they are much larger, they occupy some 70-85% of the volume. The small endothelial cells are the most abundant but occupy about 5% of the volume. As their name implies, cardiomyocytes are the heart's muscle cells but they differ from the vast majority of the body's muscle cells by being highly resistant to fatigue; while even a well-trained athlete's skeletal muscles eventually fatigue, such a performance would be very unhelpful for an organ that has to contract and relax continuously in order to ensure circulation. The cell's myofilaments, comprising the proteins myosin and actin are responsible for this contraction activity. These cells also contain large numbers of mitochondria to maintain high levels of ATP.

[31] Wang H, Leinwand LA & Anseth KS. Cardiac valve cells and their microenvironment – insights from *in vitro* studies, *Nature Reviews Cardiology,* 2014;11(12):715-27. doi:10.1038/nrcardio.2014.162.
[32] Litvinukova M. Talavera-Lopez C. Maatz, H, *et al*. Cells of the adult human heart, *Nature,* 2020;588:466-72. doi:10.1038/s41586-020-2797-4.

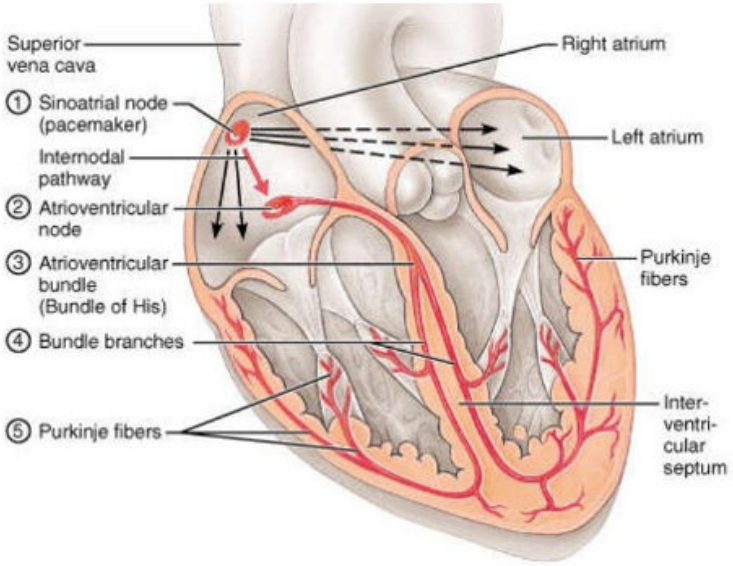

Figure 2.10. The conduction system of the human heart.

Although the function of the heart is to pump blood to all parts of the body, it should not be forgotten that cardiac tissues need their own blood supply. This is largely derived from the coronary arteries. The two main arteries originate from the aorta where it exits the left ventricle. The left coronary artery extends along the coronary sulcus, branching into the circumflex artery and the left anterior descending artery; its branches service much of the interventricular septum, the bundle of His and the anterior and lateral parts of the left ventricular wall. The right coronary artery tracks the right coronary sulcus, which leads to the right marginal artery, providing blood to the right ventricle and the AV node. Blockage of any of these coronary arteries is serious, and often fatal if untreated. The greater cardiac veins account for 95% of venous drainage, which leads to the coronary sinus, within the atrioventricular groove, emptying directly into the right atrium.

As with the brain, there are many opportunities for dysfunction of these various components of the heart, for which some form of reconstruction may be relevant.

2.2.1.2.1 Congenital heart defects

There are many different types of congenital abnormality of the heart, just the major ones being mentioned here. Tetralogy of Fallot is a condition that has been observed over several centuries, and was described, but not fully understood, by William Hunter in the 18th century; a distinguishing feature which he and others observed were the frequent spells of blue discoloration and fainting. The details of the congenital defect were ultimately described by the French physician Etienne-Louis Arthur Fallot[33], using descriptors such as '*cyanose cardiaque*' and '*la maladie bleue'*. The term tetralogy of Fallot was bestowed, in 1924, on this phenomenon by Maude Elizabeth Seymour Abbott, the renowned pediatric cardiologist in Montreal, who unsurprisingly used the French version of 'four' to describe the condition described by Fallot that had four defining features, a hole between the two lower chambers, pulmonary valve stenosis, a misplaced aorta and hypertrophied muscle in the right ventricle. Symptoms, which varied considerably, included shortage of breath, especially with exercise, clubbing of fingers and toes, and seizures.

[33] Loukas M, Cesmebasi A, Le Duong, *et a*l, Etienne-Arthur Louis Fallot and his tetralogy. *Clinical Anatomy*. 2014, 27(7): 958-63. doi:10.1002/ca22441.

Figure 2.11. Etienne-Arthur Louis Fallot, in Paris and Canadian stamp recognizing Maude Elizabeth Seymour Abbott, pediatric cardiologist, who introduced the term 'Tetralogy of Fallot'.

The so-called 'hole in the heart' in children involves a congenital hole in one of the septal walls that separate two of the chambers. A ventricular septal defect is the most common (at least in the USA) and refers to a hole in the wall between the two ventricles, while the equivalent defect in the upper chambers is the atrial septal defect. A patent foramen ovale has some similarities to the atrial septal defect but is caused by failure of the foramen ovale to close during development. In these situations, there may be few, if any, symptoms, but in severe cases, they may result in shortness of breath, fatigue, swellings of limbs, strokes, and heart murmurs. Reconstruction of the heart through some form of closure may be necessary.

Finally, there is the closely related patent ductus arteriosus. The ductus arteriosus is a fetal artery that connects the aorta and the pulmonary artery. The ductus allows blood to flow away from the lungs before birth. After birth, this is no longer needed, and it usually narrows and closes in the first few days. Failure of the ductus to close is common in premature infants and in some cases may need reconstruction.

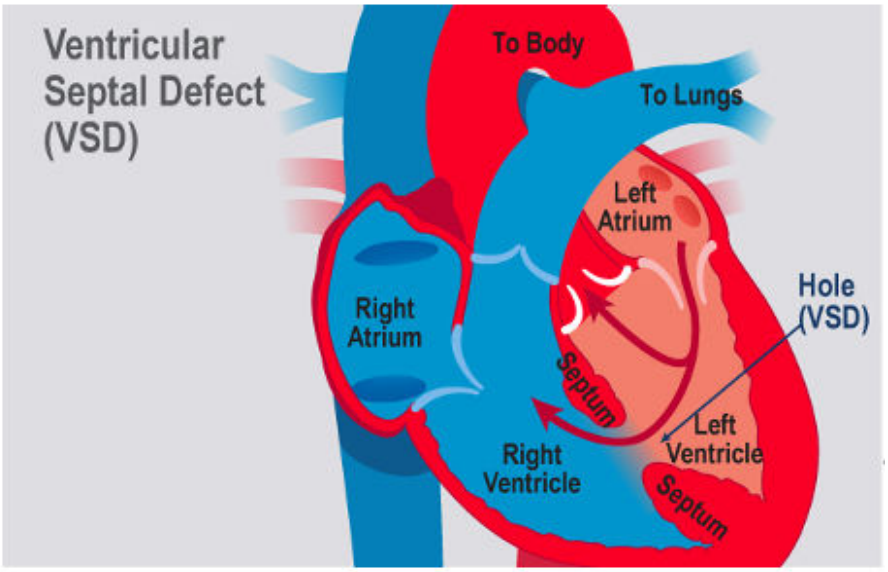

Figure 2.12. Ventricular septal defect.

2.2.1.2.2 Arrythmias

Arrythmias are irregular patterns of the cardiac electrical pulses; these may be relatively harmless but often threaten life quality and maybe fatal. A healthy heart beats between 60 and 100 times a minute. The condition of tachycardia refers to a resting heart rate greater than 100 beats per minute, while bradycardia refers to a resting rate of less than 60.

Tachycardias in the atria are of several forms. Atrial fibrillation is caused by chaotic impulses that affect the AV node in the atria; it may be transient but could result in stroke. Supraventricular tachycardia originates above the ventricles in the atria or AV node, and usually cause sudden episodes of palpitations. Ventricular tachycardia is a rapid heart rate that does not allow the ventricles to fill and contract efficiently and is often a medical emergency. Ventricular fibrillation usually affects individuals with underlying heart disease and is associated with chaotic pulses that cause quivering of the ventricles rather than solid pumping activity. Normal rhythm must be restored within a few minutes in order to prevent permanent damage or death. Long QT syndrome, often associated with genetic mutations, produces episodes of chaotic, rapid, beats, causing fainting or sudden death.

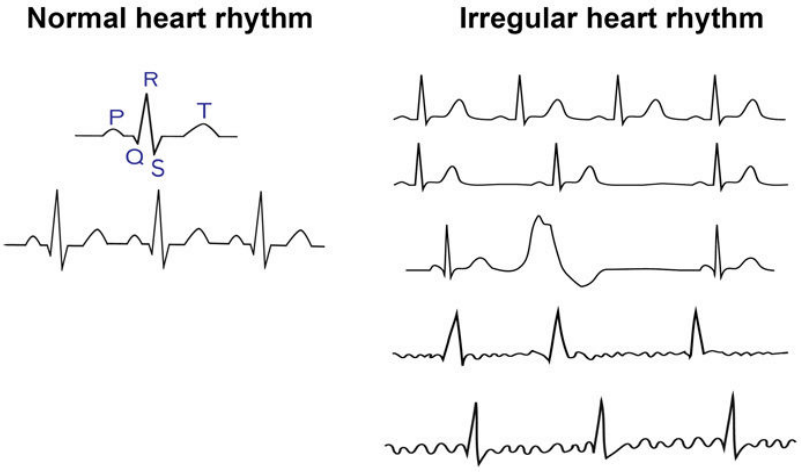

Figure 2.13. Heart rhythms.

Bradycardia is usually less serious, and most individuals can survive very effectively and asymptomatically with fewer than 60 beats per minute. A partial block of the electrical pathway near the AV node can result in significant lowering of the heart rate, which can also be caused by dysfunction of the pathways at the sinus node, giving sick sinus syndrome.

Arrythmias are often asymptomatic, or indeed undetected, and many cases can be treated pharmacologically. Physical reconstruction of the nerve conduction system is not practical and so reconstructive techniques have to be augmentative rather than replicative in nature. Thus, implanted generators of impulses can be used to override, permanently or on demand, the natural system (i.e. cardiac pacemakers), and systems, either external or fully implantable, can be used to counter tachycardia / fibrillation episodes (defibrillators).

2.2.1.2.3 Heart valve disease

As noted above, the heart beats some 3 billon times in an average life, which implies that each of the four valves must open and close, with great efficiency, this number of times. In the extreme situation with the aortic valve, the leaflet membrane stress in diastole is 2 – 3 kPa, the shear stress on the ventricular side peaks is around 7 Pa, the bending stress and strain in systole are 1.2 MPa and 15%, and the radial tensile

strain is 20%[34]. The valves, therefore, experience high strain, relatively high stress fatigue conditions for number of cycles unheard of in the performance of man-made engineering structures.

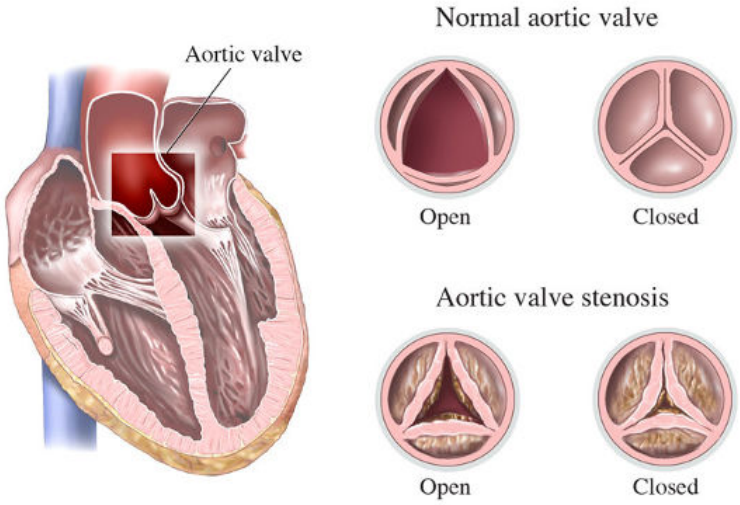

Figure 2.14. Normal and calcified / stenotic aortic valves.

Heart valve diseases may be congenital, but most of them are associated with infectious diseases or age-related structural changes. The main source of infection-related heart valve dysfunction is rheumatic fever, which is endemic in many parts of the world, but not in high-income countries. The factors associated with rheumatic heart disease (RHD) are covered in several places in this book.

Age and general health related conditions primarily include regurgitation (especially seen in the mitral valve[35]), in which the leaflets do not close properly, causing backward flow of the blood, and stenosis, in which the leaflets thicken and stiffen, reducing blood flow through the valve, especially seen in the aortic valve[36]. There are many risk factors and mechanisms for valve stenosis primarily include leaflet calcification processes. In the USA, nearly 2% of the adult population suffer some form of aortic stenosis.

Heart valve dysfunction is a prime target for replacement, but not regeneration, technologies. Some surgical procedures were introduced over a century ago in order to relieve these conditions, but it was not until the introduction of the heart – lung machine that valve replacement became possible in the early 1950s[37].

[34] Balachandran K, Sucosky P & Yoganathan AP, Hemodynamics and mechanobiology of aortic valve inflammation and calcification. *International Journal of Inflammation*. 2011, Art 263870. doi:10.406/2011/263870.
[35] Apostolidou E, Maslow AD, and Poppas A, Primary mitral valve regurgitation: Update and review. *Global Cardiology Science and Practice.* 2017;3. doi:10.21542/gcsp.2017.3.
[36] Lindman BR, Clavel M-A, Mathieu P, *et al*, Calcific aortic stenosis. *Nature Reviews Disease Primer*s. 2016;2:Art 16006. doi:10.1038/nrdp.2016.6.
[37] Russo M, Taramasso M, Guidotti A *et al*, The evolution of surgical valves. *Cardiovascular Medicine*. 2017;20(12):285-92. doi:10.4414/cvm.2017.00532.

2.2.1.2.4 Coronary artery disease (CAD)

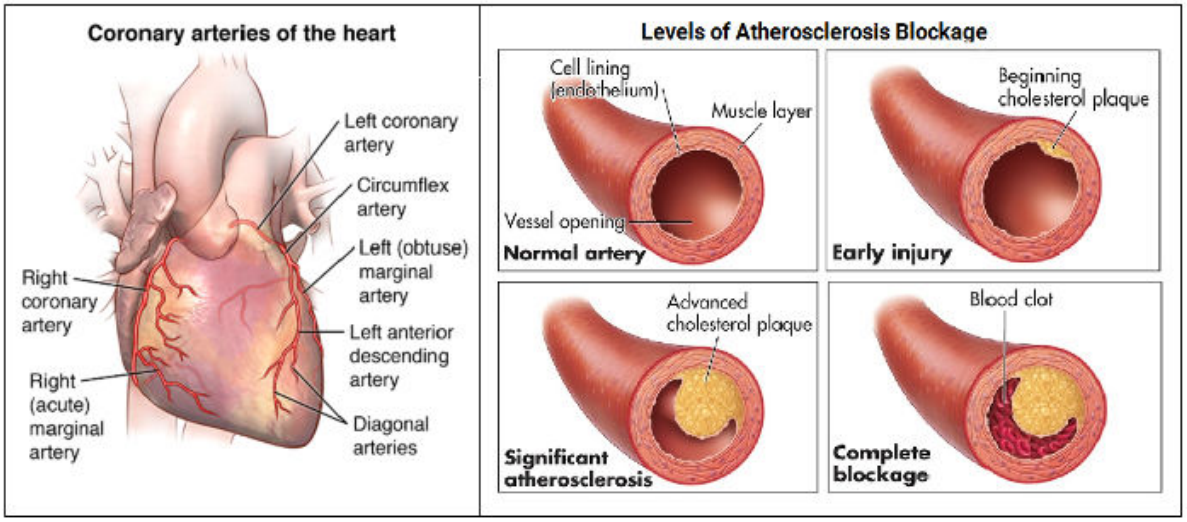

Figure 2.15. Coronary arteries and atherosclerosis.

CAD is caused by a buildup of plaque within the walls of the coronary arteries that partially or totally blocks blood flow, which is critical to heart function. The specific condition is defined as atherosclerosis; at one time this was considered to be essentially a disease of cholesterol storage but is now known to be a complex inflammatory disease[38]. It is a chronic condition, and the growth of the plaque to the point of causing stenosis represents the end stage of a pathological process affecting much of the arterial endothelium. Risk factors are age, hypertension, high blood cholesterol / triglycerides, obesity, diabetes, physical inactivity, and stress.

The main consequence of coronary artery disease is myocardial infarction, commonly referred to as heart attack. Often there are warning signs of a heart attack, such as recurrent chest pain, or angina, but often the attack is sudden and severe, with pain, shortage of breath and dizziness, all associated with the decreased flow of blood to the myocardium. During a heart attack, parts of the plaque may rupture, causing blood clotting that results in blockage and, possibly, release of emboli.

Avoidance of CAD through lifestyle modification is clearly an important factor, and some pharmacological regimes, for example with statins or aspirin, may reduce incidence, but the impact is still high, with over 15 million people over the age of 20 years in the USA developing CAD, with one-third of all deaths for those over 35 years of age being caused by the condition[39].

It is not surprising that many different engineering methods have been developed to treat patients suffering from CAD. These include techniques to disrupt or compress plaques or to replace or bypass the affected arteries. In theory, the ability to regenerate the myocardium is an attractive ides, but this has so far proved very difficult. Much attention in the arena of tissue reconstruction focuses on the arteries and the myocardium.

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[38] Libby P & Theroux P, Pathophysiology of coronary artery disease. *Circulation*. 2005, 111(25):3481-8. doi:10.1161.CIRCULATIONAHA.105.537878.

[39] Sanchis-Gomar F, Perez-Quilis C, Leischik R, *et al,* Epidemiology of coronary heart disease and acute coronary syndrome. *Annals of Translational Medicine.* 2016;4(13):256. doi:10.21037/atm.2016.06.33.

2.2.1.2.5 Heart failure

The consequence of any of the above heart conditions that are left untreated is the eventual decline in the ability of the heart to satisfactorily pump blood around the body, ultimately leading to congestive heart failure. This progressive condition may result in the chambers stretching in order to accommodate more blood, kidneys retain more fluids and lungs become congested and tissues in general swell as they also retain more fluids. Once again lifestyle changes and some drugs significantly impact the occurrence of heart failure, but the incidence is still over 23 million people affected worldwide[40]. If the primary cause of this heart failure is clearly identified, then one of the above-described therapies may alleviate the condition and prolong life. Ultimately, however, congestive heart failure is the cause of eventual death. For a very few people, solutions for reconstructing the heart may be available, including an implantable device to support left ventricular function, or a transplanted heart from a suitable donor.

2.2.1.2.6 Poetry and the heart

There is no other organ, or any other part of the body for that matter (leaving aside the erotic and pornographic), that has been subjected to the imagination of authors, poets, musicians and painters than the human heart. It would be invidious to select any of the works of the maestros or masters that adequately represent the role of the heart in human experiences. So I chose an extract of a poem written by a relatively young poet born in New York to provide a good exemplar:

My heart is bleeding. It bleeds upward and fills
my mouth up with salt. It bleeds because of a city in ruins,
the chair still warm from sister's body,
because it will all be irreproducible. My heart
bleeds because of baby bear not finding mama bear and it bleeds
to the tips of my fingers like I painted my nails Crimson.
Sometimes my heart bleeds so much I am a raisin.
It bleeds until I am a quivering ragged clot, bleeds at the ending
with the heroine and her sunken cancer eyes, at the ending
with the plaintive flute over smoke-choked killing fields. I'm bleeding
a river of blood right now and it's wearing a culvert in me for the blood. My heart
rises up in me, becomes the cork of me and I choke on it. I am bleeding
for you and for me and for the tiny babies and the IED-blown
leg. It bleeds because I'm made that way, all filled up with blood,
my sloppy heart a sponge filled with blood to squeeze onto
any circumstance. Because it is mine, it will always bleed.
My heart bled today.

***'Bleeding Heart'* by Carmen Giménez Smith, Poetry Foundation, accessed 2021**

[40] Roger VL, Epidemiology of heart failure. *Circulation Research.* 2013, 113(6):646-59. doi:10.1161/CIRCRESAHA.113.300268.

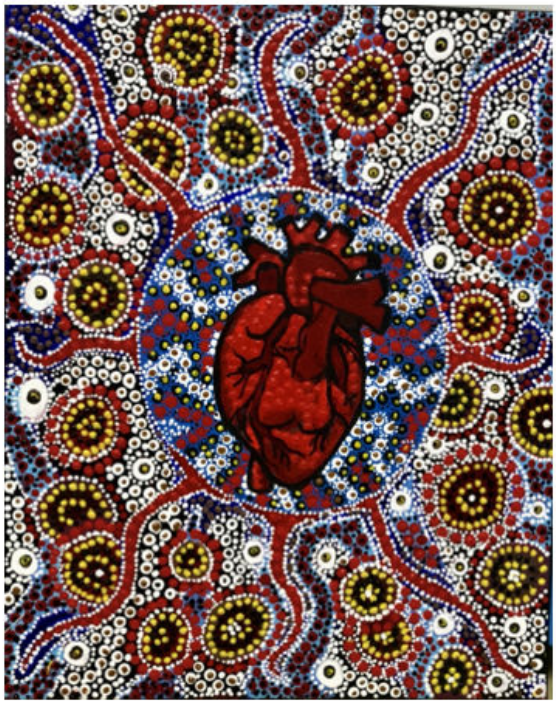

Figure 2.16. "*The Spirit of the Art"* describes Lesley Ah See's personal journey to a heart transplant. The art was painted as a gift for Lesley's cardiologist, Professor Peter MacDonald., St Vincent's Hospital[41], Sydney.

And finally, the painting above was created by a patient in an Australian hospital, presented to her cardiologist after a heart transplant.

2.2.1.3 Kidneys

The kidneys play a crucial role in human metabolism, but their function largely goes unnoticed until it goes wrong. They function at the end of the metabolic chain by orchestrating the elimination of metabolic waste.

In the process of detoxification and removal of this waste, the human kidneys filter the entire blood volume about 30 times a day[42]. Roughly 98% of this fluid is reabsorbed, producing less than 2 liters of urine per day. The kidneys operate through a combination of filtration, selective reabsorption, and active secretion processes; these mechanisms allow the kidneys to regulate fluid balance and pH, and contribute to the maintenance of blood pressure, red cell count and bone density.

Of critical importance to these functions is the architecture of the organ, especially that of the nephron, which is the essential structural and functional unit of the kidney. The nephrons are aligned with corresponding tubular sections adjacent to each other, which provides a counter-current mechanism for controlling urinary concentration and fluid / ion balance.

[41] www.svhheart.com.au.

[42] Williams DF, A systems engineering approach to restoring kidney structure and function, In, Orlando G, Remuzzi G, Williams DF, "*Kidney Transplantation, Bioengineering and Regeneration*", Elsevier, London, 2017, Ch 55, pp 769-84. ISBN 9780128017340.

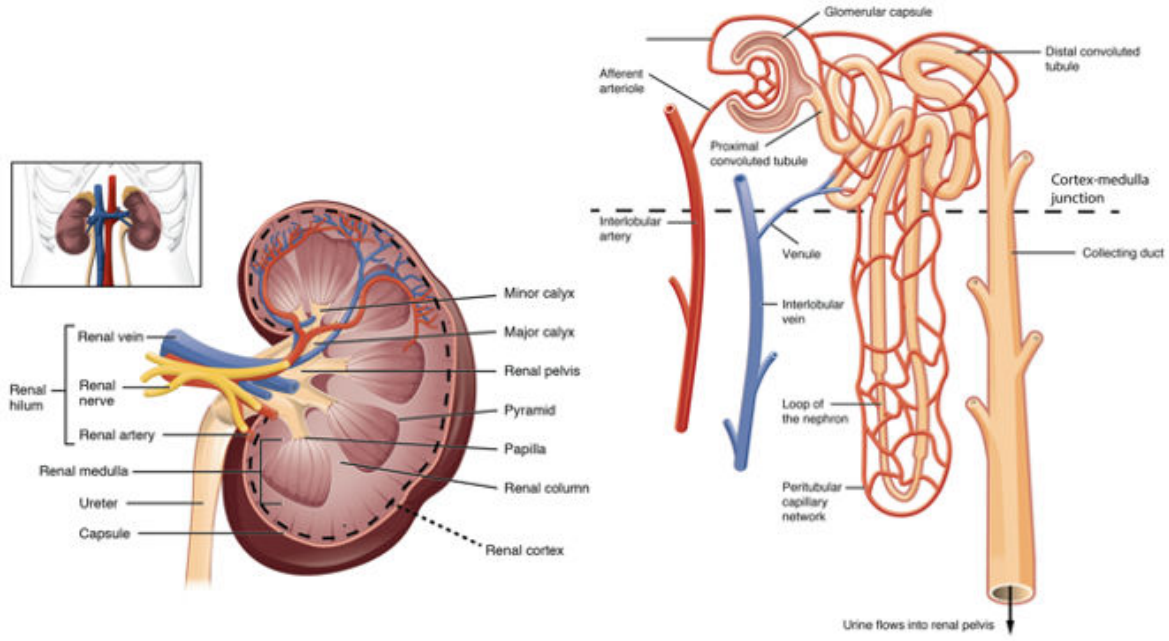

Figure 2.17. Gross architecture of human kidney (left) and nephron (right).

Within the nephron, the blood is filtered in the glomerulus and the filtrate is collected in the Bowman's capsule. The filtrate consists of water and solutes of molecular weight 60,000 or less, including urea, small proteins, amino acids, and glucose. The filtrate enters the proximal convoluted tubule where major reabsorption takes place, along with the secretion of some metabolites such as creatinine. Hormone-controlled reabsorption and secretion also takes place in the distal convoluted tubule. The arrangement of the descending and ascending limbs of the loop of Henle allows for control of the osmotic gradient.

The glomerulus and Bowman's capsule are contained within the renal corpuscle, wherein there are four different cell types, the glomerular endothelial cells (GEC), podocytes, mesangial cells, and parietal epithelial cells. The glomerular filtration barrier (GFB), which is the key selective filter, consists of the GECs, podocytes, and the glomerular basement membrane. This GFB prevents the passage of cells and high molecular weight plasma components; permeability here is influenced by the charge on the molecules, anionic macromolecules being particularly restricted. These electrokinetic characteristics are important, along with the organization of podocytes and the associated mechanical stresses.

2.2.1.3.1 Mechanisms of Kidney Failure

In contrast to the brain and the heart, where dysfunction can occur independently in one of several compartments, kidney failure is more generalized, involving the tubular system, the microvasculature or both. For many years, kidney diseases were considered to be either acute kidney injury (AKI) or chronic kidney disease (CKD), although today they are more of an integrated clinical syndrome[43]. AKI usually involves a reversible increase in serum creatinine and blood urea concentration and may be caused by glomerulonephritis (infection or autoimmunity), drug associated nephritis or vascular conditions. The progression of CKD may involve inflammatory, toxic, or physiological stress. Up to a point, such changes may be reversible through the body's inflammatory and repair processes, but if these are not tightly

[43] Chawla LS and Kimmel PL, Acute kidney injury and chronic kidney disease: an integrated clinical syndrome. *Kidney International.,* 2012;82:516-24. doi:10.1038/ki.2012.208.

regulated, they can become destructive, ultimately leading to end-stage renal disease. In a later section of this book, the ways in which bioengineering and transplantation techniques have been used to provide therapy for both AKI and CKD will be discussed as major examples of the reconstruction of the human body, but as yet these are far from satisfactory.

2.2.1.3.2 Kidneys and the arts

The kidneys and associated fluids (however described) are far less attractive to artists than the heart and blood. I refrain from even mentioning some of these 'artistic outputs' but it is worth considering the work of Michelangelo.

Figure 2.18. Michelangelo "*Separation of the Land from the Waters'*, Sistine Chapel, Rome, Italy.

Michelangelo suffered from gout, arthritis and, it is reported, from obstructive nephropathy[44]. He seems to have used his personal interest in kidney disease to influence some art, specifically his painting on the Sistine ceiling, where his third image of the series on the Creation, *Separation of Land and Water* (sometimes referred to as Separation of Earth from the Waters), features the shape of a dissected kidney. It has been inferred that this represents the separation of solids (the 'Land') from liquid (the 'Water'), perhaps also reflecting his own purification.

Michelangelo is not so well known as a poet, but he wrote more than 300 poems. Here is one, number 267, on the subject of urine and his kidneys:

> *I'm packaged in here like the pulp in fruit*
> *compacted by its peel. In lonely gloom,*
> *a genii in a jar. Dumped destitute.*
> *No room for flying high. I'm in a tomb*
> *where mad Arachne and her creepy crew*
> *keep jittering up and down, a spooky loom.*
> *My entryway's a jakes for giants, who*
> *gorge on gut-loosening grapes or suffer flux.*

[44] Eknoyan G, Michelangelo: art, anatomy and the kidney, *Kidney International,* 2000;57(3):1190-201. doi:10.1046/j.1523-1755.2000.00947x.

No other comfort station seems to do.
Urine! How well I know it—drippy duct
compelling me awake too early, when
dawn plays at peekaboo, then yonder—yuck!—
dead cats, cesspool and privy slosh, pigpen
guck—gifts for me, flung hit-or-miss?
Can't trudge to a proper dunghill, gentlemen?
Soul gets some help from body though in this:
if guts, unclogged, could ventilate their smell
no bread and cheese would keep it in duress,
while round it now catarrh and mucus jell.

Michelangelo, Poem 267 in The Complete Poems of Michelangelo, Translated by John F. Nims [45].

2.2.1.4 Liver

2.2.1.4.1 Poetic and spiritual aspects of the liver

The liver is deceptive. When we see a piece of lamb's liver in the butcher shop, it looks like an unexciting, homogeneous, monotonic, blob of tissue. Yet it's ultrastructure and microarchitecture are extraordinarily complex and this mass (it is the largest organ in the body) performs many varied and consequential functions. To confirm this point I start, rather than end, with artistic and spiritual aspects.

The Chilean Poet, Pablo Neruda decided to write a series of 'elemental odes', including poems about the body's organs. He was first advised to concentrate on the brain but was convinced by a hepatologist to write about the liver, hence Neruda's 'Ode to the Liver'[46]. The persuasive argument was that '*the liver is a modest organ, so little mentioned, so hidden despite having such a fundamental role. To celebrate the brain, which has given human beings their arrogance, would be prosaic. With an ode to the liver, you would be drawing back a veil, mending an injustice*'. Part of the English translation of this poem (re-formatted to give some style since the original had just one or two words per line), follows:

Modest, organized friend, underground worker,
Let me give you the wing of my song,
The thrust of the air, the soaring of my ode.

It is born of your invisible machinery,
It flies from your tireless confined mill,
Delicate powerful entrail, ever alive and dark.

While the heart resounds and
Attracts the music of the mandolin
There, inside, you filter and apportion,

You separate and divide, you multiply and lubricate
You raise and gather the threads and the grams of life
The final distillate, the ultimate essence.

[45] 'The Complete Poems of Michelangelo', Translated by Nims, JF, University of Chicago Oress, 1998.
[46] Arrese M (Editorial), The liver in poetry: Neruda's 'Ode to the Liver, *Liver Int*ernational. 2008; 901-3. doi:10.1111/j.1478-3231.2008.01814.x.

Submerged viscus, measurer of the blood,
You live full of hands and full of eyes
Measuring and transferring in your hidden alchemical chamber,

Yellow is the matrix of your red hydraulic flow,
Diver of the most perilous depths of man,
There forever hidden, everlasting in the factory, noiseless.

Extract from Pablo Neruda, '*Ode to the Liver*', translated by Oriana Josseau Kalant, 2003

As noted elsewhere in this book, according to Traditional Chinese Medicine (TCM), all internal organs are divided into two major categories, the 5 zang and 6 fu organs. The liver is included in the former[47]. The main function of zang organs is to store jing-qi, i.e., 'essence', the refined food nutrient responsible for maintenance of life's activities. The fu organs transport and transform food. The liver is responsible for 'dispersion and dredging', for the regulation of emotion, the promotion of digestion and absorption, the maintenance of qi, blood and body fluid, and reproductive function. The liver is the basis of spiritual consciousness through hun, which especially motivates our higher desires and our passions. Among other properties, the liver is related to anger and to the source of endurance; the liver governs tendons and opens into the eyes. Generally, the liver, and especially the liver meridian, has a more prominent role in health according to TCM than accepted in western medicine; liver activity is based upon the morphological characteristics of the organ, but, *via* the liver meridian, it is connected to the hypothalamus, reticular structure, limbic system, visual and auditory pathways and many more structures.

2.2.1.4.2 Structure and function of the liver

The liver receives oxygenated blood from the hepatic artery and deoxygenated blood from the portal vein; it, therefore, acts as a barrier between the digestive tract and the rest of the body, transforming, detoxifying, and accumulating metabolites. It also produces several types of plasma protein, such as albumin. It is composed of polygonal lobules that are separated by connective tissue. At the periphery of the lobules are areas of these arteries and veins along with bile ducts, lymphatic vessels and nerves. At the center of the lobules is the central vein. Hepatocytes are the main structural components of the liver, occupying some 80% of liver volume, and they are arranged radially within the lobules to form cellular plates, between which are liver capillaries and sinusoids, and within which are sinusoidal cells, Kupffer cells, stellate cells, and hepatic NK cells. The blood from arterial and venous sources mingles in the sinusoids, serving important filtering functions, including the removal and processing of antigens derived from both the systemic circulation and the gastrointestinal tract, implying a central role in the body's immune system.

[47] Liu Z-W, Shu J, Tu J-Y*, et al*, Liver in the Chinese and Western Medicine. *Integrative Medicine.* 2017;4:39-45. doi:10.1159/000466694.

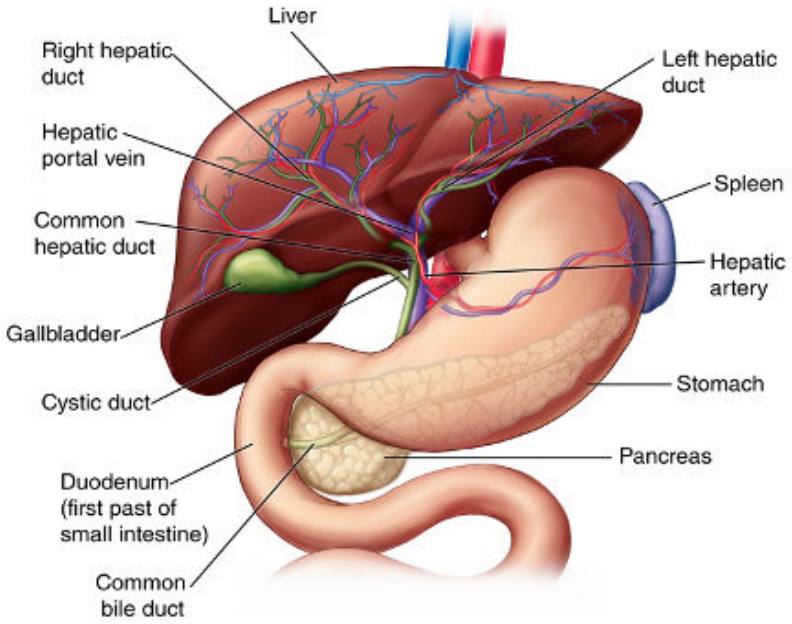

Figure 2.19. Anatomy of the human liver.

2.2.1.4.3 Liver Diseases

The global prevalence of non-alcoholic fatty liver disease (NAFLD) is estimated at 25%[48]; it encompasses the entire spectrum of disease that is characterized by hepatic steatosis (accumulation of fat), and effects caused by excessive alcohol consumption, hereditary conditions, and iatrogenic causes. Although the early stages may be difficult to diagnose, and could be reversed with lifestyle changes, especially related to obesity factors, for most people it is progressive, eventually leading to irreversible nonalcoholic steatohepatitis (NASH). The pathogenesis is complex and multifaceted, progression being closely related to metabolic syndrome, the cluster of conditions that often occur in the context of heart disease, stroke, and type II diabetes. Obesity-related inflammation and insulin resistance are among many related factors, and the end result is either cirrhosis or hepatocellular carcinoma. In terms of therapy, resection of parts of the liver can have positive effects on carcinoma, but liver transplantation is usually the only option for extensive carcinoma. There are, of course, may risk factors associated with liver disease, excessive consumption of alcohol being pre-eminent, but also viruses, especially hepatitis A, B or C.

2.2.1.5 Pancreas

The pancreas lies transversely between the duodenum and the spleen. It is divided into four parts, the head, neck, body, and tail. The head lies on the inferior vena cava and renal vein and is surrounded by a loop of the duodenum. The tail extends to the splenic hilum. Branches of the splenic artery, superior mesenteric artery and the common hepatic artery provide the blood supply, while the head drains into the superior mesenteric vein and the body and tail drain into the splenic vein. The majority of the organ consists of exocrine tissue, made of pancreatic acini in which are contained a variety of enzymes and receptors for neurotransmitters that regulate exocytosis of the digestive enzymes. Crucially, the pancreas

[48] Kaufmann B, Reca A, Wang B, *et al,* Mechanisms of non-alcoholic fatty liver disease and implications for surgery. *Langenbeck's Archives of Surgery*. 2021;406:1017. doi:10.1007/s00423-020-01965-1.

also houses the islets of Langerhans, which contain endocrine cells, including beta cells that produce insulin.

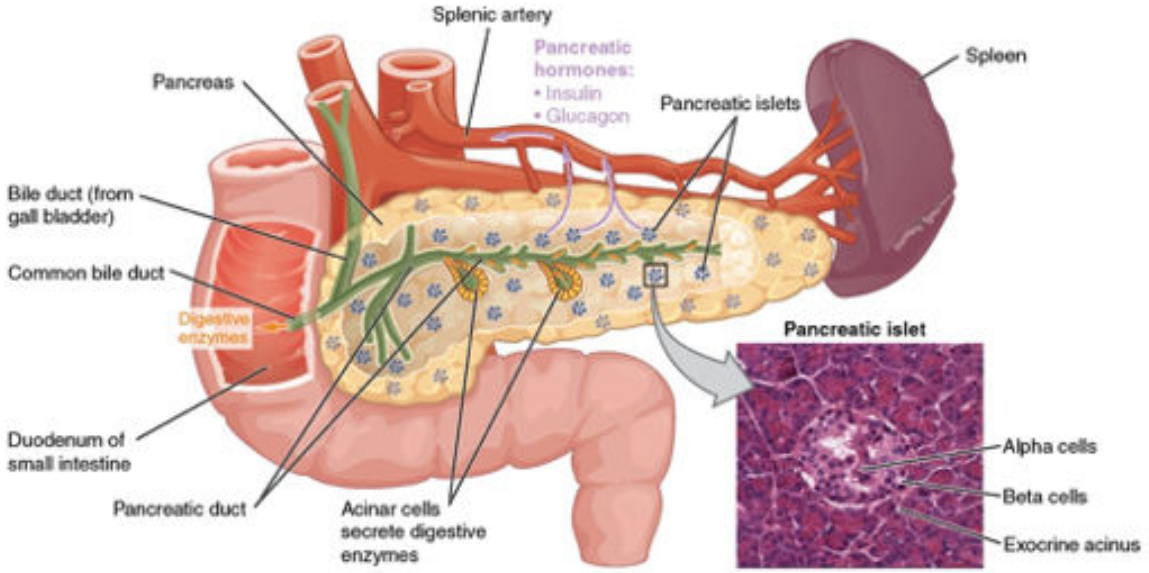

Figure 2.20. Structure of human pancreas.

There are some lesser conditions that affect the pancreas, such as perforation. The three significant conditions are pancreatitis (an intensely painful inflammation), pancreatic cancer, which is very difficult to treat and is usually diagnosed too late for any effective therapy (the average age of diagnosis being 71), and diabetes mellitus. This latter condition will be discussed elsewhere since it has been a long-established, but yet unsuccessful, target for regenerative medicine; briefly diabetes mellitus type 1 is an autoimmune condition that results in decreased production of insulin, while diabetes mellitus type 2, the most common form, causes high blood sugar levels due to a combination of insulin resistance and impaired insulin secretion.

2.2.1.5.1 Spirituality aspects of diabetes management

Diabetes affects patients at both ends of the age spectrum, and a whole variety of psychological and spiritual factors play a role in the management of patients; the increasing incidence of both types of diabetes suggests that attention should be paid to these aspects. With pediatric or adolescent cases, the realization that self-management of the condition is a lifetime commitment is a major event in most of the lives of these patients. One recent study in Sweden showed that the risk of psychiatric morbidity in children with diabetes type 1 compared with the general population was tripled within 6 months of onset, and an increased risk was noted with suicide attempts[49]. Very few studies or analyses of this situation have been carried out, perhaps not surprisingly in view of the intractability of the problem. In addressing the role of spiritual intelligence in self-management in adolescent type 1 diabetic patients in a

[49] Butwicka A, Frisen L, Almqvist C, *et al,* Risks of psychiatric disorders and suicide attempts in children and adolescents with type 1 diabetes: A population-based cohort study. *Diabetes Care.* 2015;3:4539. doi:10.2337/dc14-0262.

predominantly Muslim environment in Iran, Rahmanian *et al*[50] considered that those with spiritual beliefs were more adaptable in accepting their illness, strengthening spiritual intelligence being used as a means of compromising disease, managing blood glucose, and controlling complications. It has to be said that the statistical analysis in this paper, as indeed suggested by the authors themselves, was somewhat equivocal, which reflects the difficulty mentioned above, but despite this, the point is well made. At the other end of the age spectrum, limb amputation due to diabetes can cause crises in the spiritual life of individuals[51]. From research also conducted in a Muslim community, spiritual health was found to be the unique element that can harmonize physical, mental and social aspects of the condition.

Many professional and patient support groups strongly emphasize the role of mental health in the holistic approach to diabetes case, such as Diabetes UK, as shown below:

Figure 2.21. Mental health and holistic care of diabetes[52].

[50] Rahmanian M, Hojat M, Fatemi NS, *et al*, The predictive role of spiritual intelligence in self-management in adolescents with type 1 diabetes. *Journal of Education and Health Promotion,* 2018;7: 69. doi:10.4103/jehp.jehp_182_17.

[51] Salehi S, Ghodousi A and Ojaghloo K, The spiritual experiences of patients with diabetes-related limb amputation, *Iran Journal of Nursing and Midwifery Research*, 2012, 17(3):225-8.

[52] www.diebetes.co/uk.

2.2.1.6 Lungs

The lungs**,** the largest organs of the respiratory tract, are suspended within the pleural cavity of the thorax. They are surrounded by thin membranes, the pleura, which secrete a fluid that allows the lungs to move freely during expansion and contraction in breathing. The lungs are divided into lobes, the right lung containing three lobes and the smaller left lung containing two. The alveoli, which are very small air sacs, are the functional units of the lungs where gas exchange takes place. The two lungs may contain as many as 700 million alveoli. The lungs receive blood from two major sources. The right ventricle pumps deoxygenated blood to the lungs through the pulmonary arteries, one vessel to each lung; each of these undergoes division to produce superior and inferior branches. The capillary networks within the alveolar walls coalesce distally to form the pulmonary venous circulation, where low-pressure vessels take oxygenated blood back to the left atrium. The lungs also receive oxygenated blood from the heart, *via* bronchial arteries, that provides oxygen for cellular respiration. The alveolar region of the lung constitutes about 90% of the total volume, the remainder being occupied by conducting airways and larger blood vessels[53]. Clusters of alveoli are arranged in functional units, the acini, of which there may be 30,000 per adult human lung. The inter-alveolar septum gives the structural basis for gas exchange, separating the alveolar airspace from the capillary lumen. To permit efficient gas exchange, this septum is about 2 microns thick. Respiratory mechanics depend on lung compliance, chest wall compliance, respiratory rate, and airway resistance, which work in conjunction to create negative pressure within the lungs and pleural space, allowing air to be drawn into the lungs. Conversely, a decrease in lung volume increases pressure in the lungs, forcing air out.

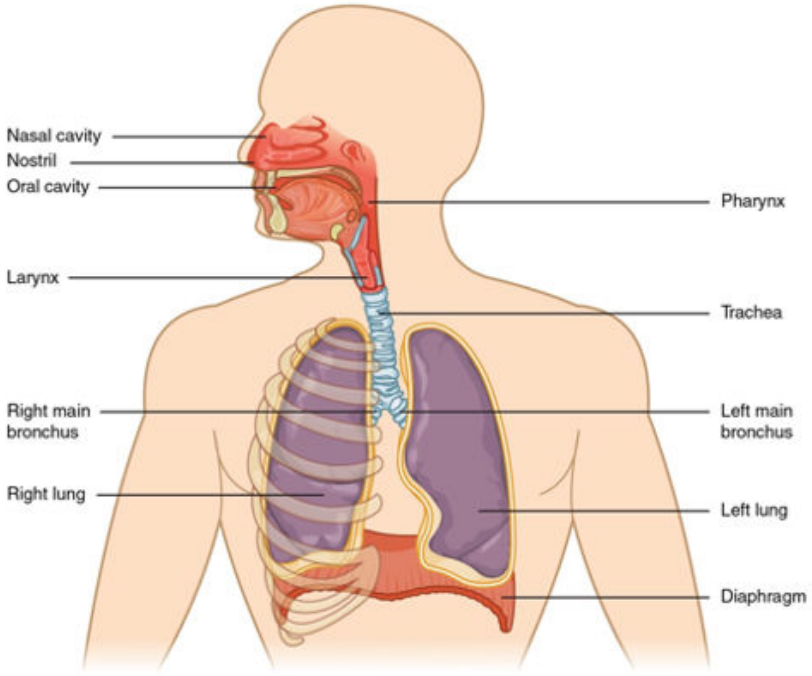

Figure 2.22. Major respiratory structures in the human.

[53] Knudsen L and Ochs M, The micromechanics of lung alveoli: structure and function of surfactant and tissue components. *Histochemistry and Cell Biology*. 2018;150:661-76. doi:10.1007/s00418-018-1747-9.

2.2.1.6.1 Lung diseases

The whole of the respiratory tract is susceptible to disease. I mention just those which directly affect the lung here, discussing the rest of this tract later, although the distinction may not always be clear. Lung diseases or disorders can affect respiratory function i.e., the ability to breathe, and pulmonary function, or how well lungs work. Some of these diseases are caused by bacterial, viral, or fungal infections, while others are associated with environmental factors, including asthma, mesothelioma, and lung cancer. Chronic lower respiratory diseases, a leading cause of death in the United States, is a set of conditions that includes chronic obstructive pulmonary disease (COPD), emphysema, and chronic bronchitis. Other lung conditions include pulmonary fibrosis, a lung tissue scarring that can be caused by different factors, and pneumonia, a bacterial or viral infection in which air sacs fill with fluid. Lung cancer is still very common. The two main types of lung cancer (small cell and non-small cell) grow and spread in different ways, and each type may be treated differently. Cigarette smoking is the overall leading cause of lung cancer. Other environmental factors linked to lung disease include asbestos, radon gas, air pollution, and chemicals such as uranium, beryllium, vinyl chloride, and arsenic. Lung disease caused by inhalation of foreign agents is referred to as pneumoconiosis.

Reconstructive techniques for the lung have so far been extremely difficult to establish. Lung transplantation is sometimes an option. For very limited periods of time, extracorporeal oxygenation may be used (for example as part of operative procedures when normal lung and heart functions have to be temporarily replaced), but this hardly constitutes a phase of 'reconstructing the body'.

2.2.1.6.2 Spiritual aspects of the lungs and breathing

It should not be surprising that spirituality is powerfully connected to the lungs and breathing, since 'spiritus' in Latin means 'breath or breathing'. Because of the limited interactions between conditions of the lungs and reconstructive medicine, I will not dwell on this connectivity. However, the Hebrew book of Genesis describes God breathing life into the dust (see below) and forming a living soul, and most other faiths give similar primacy to the breath of life.

Figure 2.23. Job 12:9-10 *"Who among all these does not know that the hand of YHWH has done this? In whose hand is the soul (nefesh) of every living (chai) thing, and* ***the breath (ruakh)*** *of all mankind?"*[54]

[54] https://hebrewwordlessons.com/2018/01/28.

2.2.1.7 Spleen

The spleen is a small organ in the upper left side of the abdomen, next to the stomach. It is an important part of the immune system, but survival is quite possible without it, since the liver can take over many of the spleen's functions. There is, therefore, little need to discuss the spleen in any treatise on restructuring the body. Moreover, for most people, the spleen is not a favorite organ. It has been considered the seat of anger in the human body since medieval times. The idiom 'to vent one's spleen', which has been in use for centuries, means to air one's grievances, to express anger.

The nineteenth century French poet Charles Baudelaire felt that poetry must evoke the artificial and paradoxical aspects of life. He thought that beauty could evolve on its own, irrespective of nature. But the spleen signifies everything that is wrong with the world: death, despair, solitude, murder, and disease. In contrast, the ideal beauty of life represents a transcendence over the harsh reality of spleen and is taken up in much of his poetry. Baudelaire often uses erotic imagery to convey the impassioned feeling of the ideal but is consistently disappointed as the spleen again takes up its reign. This is seen in the following extract from 'Spleen":

I'm like the king of a rainy country, rich
but helpless, decrepit though still a young man
who scorns his fawning tutors, wastes his time
on dogs and other animals, and has no fun;
nothing distracts him, neither hawk nor hound
nor subjects starving at the palace gate.
His favorite fool's obscenities fall flat
—the royal invalid is not amused—
and ladies in waiting for a princely nod
no longer dress indecently enough
to win a smile from this young skeleton.
The bed of state becomes a stately tomb.
The alchemist who brews him gold has failed
to purge the impure substance from his soul,
and baths of blood, Rome's legacy recalled
by certain barons in their failing days,
are useless to revive this sickly flesh
through which no blood but brackish Lethe seeps.

—And giant hearses, without dirge or drums,
parade at half-step in my soul, where Hope,
defeated, weeps, and the oppressor Dread
plants his black flag on my assenting skull.

Extract from *'La Spleen de Paris'* by Charles Baudelaire[55]

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[55] Charles Baudelaire, "*La Spleen de Paris"*, Version published by Georges Cres et Cie, Paris, 1917.

2.2.1.8 Sensory Organs

2.2.1.8.1 The eyes

It is self-evident that the function of the human eye is to allow individuals to see their environment. Sight, as many a poet, author, playwright, philosopher, and artist have recounted, is a spectacular success of nature and evolution.

> *"Many are the things that man seeing must understand. Not seeing, how shall we know what lies in the hand of time to come?"*
>
> **Sophocles, '*Ajax'*, [56]**
>
> *O loss of sight, of thee I most complain! Blind among enemies, O worse than chains, dungeon, or beggary or decrepit age".*
>
> **John Milton, '*The Blindness of Samson'*, [57]**

In a discussion of both the science and spirituality of humans, it is necessary to point out that sight is not the same as vision, again as many have pointed out:

> 'The only thing worse than being blind is having sight but no vision'.
>
> **Attributed to Helen Keller.**
>
> 'The real voyage of discovery consists, not in seeking new landscapes, but in having new eyes'.
>
> **Marcel Proust, In 'La Prisonniere'.[58]**

There are several anatomical and structural features that contribute to the functionality of sight. The first are those structures that allow light to pass from the body's exterior to the detection system. Secondly, there is the detection system itself which transduces light signals into electrical signals and transmits these to the brain. The third is the protection system that surrounds the eye, bearing in mind that, of necessity, the eyes are in a prominent position at the front of the head, susceptible to trauma and damage. The final structures are those associated with blood supply. These systems are tightly integrated.

For the optical functionality, the eye can be divided into five parts, the outer cornea through which light enters the eye, the anterior chamber with associated pupil and iris, the posterior chamber, the lens, and the vitreous chamber.

The cornea has five layers; the epithelium is the outer layer, around 6 cells thick, which is turned over every week; the Bowman's layer, a dense but thin sheet of fibrous connective tissue; the corneal stroma, a regular arrangement of collagen fibrils and lamellae; Descemet's membrane, 15 microns thick in adults; the corneal endothelium, a single cell inner lining. The cornea provides a significant fraction of the focusing power of the eye; any sub-optimal curvature leads to refractive errors, an important

[56] Sophocles, '*Ajax',* translation by George Thedoridis, 2009, Published by Poetry in Translation
[57] John Milton, 'The Blindness of Samson', Paradise Regained, A Poem in IV Parts, Samson Agonister, London, 1671.
[58] Marcel Proust, 'La Prisonniere', In "Remembrance of Things Past", Grasset and Gailimard, Paris, 1913-1927

characteristic that provides the rational for many reconstructive procedures of the eye. There is much to go wrong in the cornea, including abrasions, ulcers, dryness, dystrophy, and infections (acanthamoeba keratitis). The anterior chamber is filled with aqueous humor and averages about 3 mm in depth. This fluid is constantly circulated; if the trabecular network through which this flow becomes blocked, or if there is an excess of humor in the chamber for any other reason, intraocular pressure increases, which may lead to the serious condition of glaucoma. At the back of the anterior chamber, are the uvea and pupil. The uvea consists of the iris, which is pigmented giving everyone a distinctive appearance, the ciliary body and the choroid, which surrounds the whole of the posterior part of the eyeball, sandwiched between the sclera and the retina, supplying nutrients to the latter *via* its microvasculature. The pupil is the black circle in the center of the iris; it is this which regulates the amount of light entering the eye.

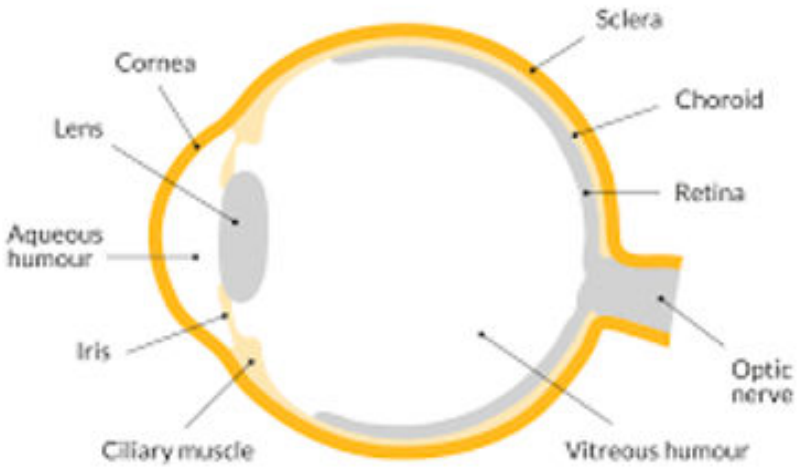

Figure 2.24. Parts of the eye.

Just behind the iris is the lens, a proteinaceous crystalline structure that is able to focus incoming light onto the retina. Ciliary muscles cause changes of shape of the elastic lens, which enables the eye to alter focus very readily, a process known as accommodation. The lens may lose some elasticity on ageing, giving the condition of presbyopia. Proteins in the lens may also clump together, resulting in cloudiness, causing cataracts to form. The vitreous chamber is that space between the lens and the retina and is filled with vitreous humor; this helps the eye keep its spherical shape and maintains the position of the retina.

The light detection and transmission system involves the retina and optical nerve. The retina is the innermost layer of the tissues found at the surface of the posterior part of the eye. The outer layer is the sclera, which is the supporting structure of the eye, and is continuous with the clear cornea at the front. It is colored white. The choroid is the vascular layer of the eye, located between the retinal pigment epithelium (the RPE) and the sclera. The primary function is to supply oxygen and nourishment to the outer retina. Additionally, it plays a significant role in the regulation of intraocular pressure. The RPE is the outermost layer of the retina, being a single layer of cells that regulate nutrition and waste removal. Inside the RPE are the photoreceptors, which convert light into signals that stimulate biological processes. The two classic photoreceptor cells are rods and cones; rods primarily contribute to night-time vision whereas cones are effective for day-time vision. Photoreceptor cells are typically arranged in an irregular but approximately hexagonal grid, known as the retinal mosaic. The human retina contains about 120 million rod cells, and 6 million cone cells. The number and ratio of rods to cones varies among species,

dependent on whether an animal is primarily diurnal or nocturnal. There are major functional differences between the rods and cones. Rods are extremely sensitive and can be triggered by a single photon such that at very low light levels, vision is based solely on the rod signal. Cones require much brighter light to produce a signal.

The macula is a 5mm wide central part of the retina which has a very high density of photoreceptors, and is responsible for central vison, fine detail, and color perception. Inside the photoreceptors are layers of horizontal and bipolar cells which help integrate the electrical signals, which are transmitted to the retinal ganglion layer, which consists of ganglion cells that collectively transmit information to the optic nerve. The optic nerve is part of the central nerve system; it is ensheathed in the meningeal dura, arachnoid, and pia mater layers. The fibers from the retina run along the optic nerve to nine primary visual nuclei in the brain, from which a major relay inputs into the primary visual cortex. These tracts have only limited regenerative capabilities so that optic nerve damage can result in irreversible blindness.

Much of the protection for an eye is provided by the orbit. This is a bony structure, which also contains extraocular muscles, vasculature, nerves, the lacrimal gland, and fat deposits[59]. The structure of the orbit is usually considered as having four elements, the orbital roof, the lateral wall, the medial wall and the orbital floor, details of which need not concern us here. The medial wall has two distinct openings in which are located the arteries and veins, and various fissures allow passage of nerves. There are 6 extraocular muscles that control eye movement. The optic nerve exits the posterior wall, traveling posterior and medial to the optic canal.

The eye is of such complexity that it is not surprising that it is subjected to a wide variety of diseases, abnormalities and illnesses, many of which may be addressed by some form of reconstruction. The most serious of these are age-related macular degeneration, cataracts, retinitis, glaucoma, ocular hypotension, retinal detachment and uveitis, all of which are considered in later chapters. To this list must be added refractive vision problems such as myopia (nearsightedness), hyperopia (farsightedness), astigmatism and presbyopia (difficulty reading small print) which can be corrected to greater or lesser extents by technologies and clinical interventions.

The eye has also played a pivotal role in spirituality and mythology over several millennia. One of the most interesting aspects here refers to The Eye of Horus[60]. Ancient Egyptians discovered many anatomical features and often documented their findings by combining mythology with mystical facts. The Eye of Horus mythology has its basis in the story of Osiris, the oldest son of the God of the earth and the goddess of the sky. Set (or Seth or Suetekh), younger brother of Osiris, murdered him to claim the throne. He dissected the body into 14 parts which were distributed across Egypt. Isis, sister of Osiris and her son Horus, sought and found 13 of these parts, allowing Osiris to pass into and rile the underworld, while Horus killed Set and reclaimed the throne. The fight was considered as a metaphor for the battle between order and chaos, Horus then being idolized. The Eye of Horus was used as a sign of prosperity, and artistically represents a remarkable likeness to neuroanatomic features that we know today. The Eye of Horus is divided into six parts called the Heqat Fractions, it being suggested that these represent each of the six senses; smell, sight, thought, hearing, taste and touch. The pupil of the eye (* in the following figure) was given the 1/4 Heqat Fraction and is the center of vision.

[59] Wilkinson MJ, Anatomy of the human orbit. *Operative Techniques in Otolarynology*, 2018;29:186-192. doi:10.1016/j.otot.2018.10.002.

[60] ReFaey K, Qioones GC, Clifton W, *et al*, The Eye of Horus: The connection between art, medicine, and mythology in Ancient Egypt, *Cureus.* 209;11(5):e4731. doi:10.7759/cureus.4731.

Figure 2.25. The representation of vision in the Eye of Horus, with a central round-shaped object that resembles the shape and location of the massa intermedia, the symbol of vision.

2.2.1.8.2 Ears

The shapes of ears, and any malformations associated with them, are easily discernable features of an individual and, accordingly, they always have been the subject of psychological / societal perceptions, from admiration to ridicule, often being associated with mystical qualities. This is not just a modern, vanity-based, phenomenon, but one of significant historical relevance[61]. So-called ear tablets dating from 1500 BC depict Egyptian perspectives on ear shape, and documents found in Thebes state that 'the breath of life enters by the right ear and the breath of death by the left ear'. During the Byzantine period, only a perfect man could claim the throne, leading to the custom of cutting off ears as a punishment for lesser crimes so that the individual could never aspire to the throne.

In oriental Buddhism and eastern Asia, large ears are taken as auspicious because they signify wisdom and compassion. The Buddha, who was born as a prince in Nepal, initially lived within a culture where wealth was very important, and his ears were adorned by large, heavy jewelry. When he renounced this culture, his ears were already long, and on becoming The Buddha, the enlightened one, the compassionate one, he was depicted with very long ears and the ability to hear the sound of the world.

Figure 2.26. The Buddha, depicted with very long ears.

[61] Gamatsi IE, Nikolopoulos TP and Lioumi DE, The ear and its malformations: strange beliefs and misconceptions, *The British Associations of Plastic Surgeons*, 2003;56:369-374, doi:10.1016/S0007-1226(03)00192-9.

The visible external ear, known as the pinna, forms only a small part of the ear. Inside the skull, surrounded by temporal bones, are the middle ear and the inner ear, which collectively control hearing and balance. The main features of these structures are the ear canal, the tympanic membrane, the middle ear components of ossicles, round and oval windows and Eustachian tube, and the inner ear components of cochlea, semi-circular canals, and vestibule. The main route for sound transmission is air conduction from the pinna to cochlea *via* these various structures; it should be noted, however, that a small component involves bone conduction, where sound is transmitted by vibrations in the temporal bone and associated cartilage and soft tissues, with resulting compression and expansion of the cochlear boundary.

In air conduction, sound waves travel through the ear canal and cause vibrations in the tympanic membrane (the ear drum), a fibrous connective tissue structure of three layers, and divided into two parts, the pars flaccida and pars tensa. The inner surface is attached to the first of the auditory ossicles, the malleus, which leads to the incus and then the stapes. The connection between the malleus and the ear drum is a lever-like hinge, while the incus and stapes are fused together (in humans and most mammals). The stapes connects to the cochlea via an opening, the oval window. The middle ear is linked to the nasopharynx by the Eustachian tube, which is normally closed but can open to allow equilibration of pressure in the gas-filled middle ear. The inner ear is filled with fluid, the endolymph in the inner parts and perilymph in the outer parts. The semicircular canals and vestibule both assist in equilibrium and balance.

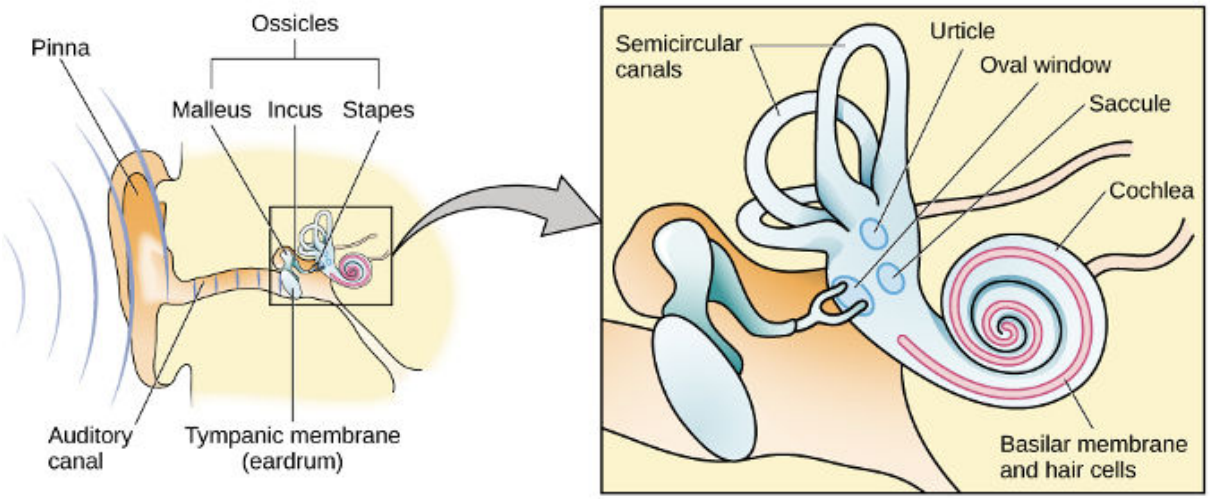

Figure 2.27. Structure of the human ear and sound conduction.

The cochlea is the critical functioning part of the ear; it consists of a coiled labyrinth, with about 2.5 turns in the human. It has three main chambers, the scala vestibuli at the top, which is separated from the scala media by a thin flexible partition, which is itself separated from the scala tympani at the bottom by a membrane that includes the basilar membrane. The organ of Corti sits on top of this membrane and contains two types of hair cells, which have stereociliary hairs projecting from their top. A difference in pressure within these chambers drives the basilar membrane into motion, the movement of the stereocilia being converted into chemical signals that excite adjacent nerve fibers, and neural impulses are transmitted along the auditory nerve to the brainstem. This whole system is remarkably sensitive and

selective; the internal motion that triggers this transformation to neural impulses can be as low as 0.3 nm, but the dynamic range is 120 dB with a resolution of 0.5 dB and a frequency range of up to 10 octaves[62].

As with the eye, there is much to go wrong in the complex microarchitecture and exquisite functionality of the ear. Leaving aside the esthetic aspects, ear disorders can involve the passage of sound waves to the inner ear, or with the detection, conversion, and transmission of impulses within the inner ear itself. Causes can be hereditary, infections (including meningitis), trauma, exposure to loud noises and ageing. Reconstructive technologies include hearing aids, which amplify sound as it enters the ear, ossicular replacement, and cochlear implants, which can replace the function of the cochlea in the profoundly deaf.

The overwhelming connection between spiritualty and hearing is found within the context of hearing spiritually significant voices[63]. This should be considered as distinct from the Auditory Verbal Hallucination concept, one of the hallmark symptoms of psychosis[64], which is associated with disorganization of speech capacity and 'voices' in deaf patients that relate to the message rather than the sound of it. The significance of this is that many people who hear voices are diagnosed with mental disorders and have negative emotional experience with the voices, but others experience voices positively, having control over the experience and interpreting them in a spiritual or religious context. This has become a very important subject, with many excellent papers and books published[65]; however, 'therapies' for the phenomena are rarely discussed, and reconstructive technologies appear to be quite inappropriate.

2.2.1.8.3 Tongue

The tongue is a small, simple, organ, but it has multiple functions; indeed, Shakespeare considered that the tongue was pre-eminent, for without it there could be no communication:

> *"He hath a heart as sound as a bell, and his tongue is the clapper: for what his heart thinks, his tongue speaks".*[66]

The tongue has eight interwoven striated muscles that are able to move in all directions, giving it considerable flexibility. It is anchored in the front of the mouth by the frenum and to the back of the mouth by the hyoid bone. It is covered with moist, pink-colored mucosa; on the upper surface it is covered with many papillae, which give it a rough texture, and which contain taste buds. The tongue is highly vascular, with four main arteries: the suprahyoid, dorsal lingual, sublingual, and deep lingual arteries. Motor innervation to the majority of intrinsic and extrinsic muscles is provided by the hypoglossal nerve. General sensation to the anterior part of the tongue is achieved through the lingual nerve and to the posterior part through the glossopharyngeal nerve.

The functions of the tongue are primarily those of taste, speech and manipulation of food. Taste buds are groups, between 50 and 100 in number, of bipolar neuroepithelial cells, mostly located in the tongue but with a minor presence on surfaces of the palate, epiglottis, pharynx and larynx. On the anterior part of the

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[62] Elliott SJ and Shera CA, The cochlea as a smart structure, *Smart Material Structures*, 2012;21(6):064001. doi:10.1088/0964-1726/21/6/064001.

[63] Cook CCH, Powell A, Alderson-Day B, *et al,* Hearing spiritually significant voices: A phenomenological survey and taxonomy. *Medical Humanities,* 2020;1-12. doi:10.1136/medhum-2020-012021.

[64] Mitropoulos GB, Auditory verbal hallucinations in psychosis: Abnormal perceptions or symptoms of disordered thought. *The Journal of Nervous and Mental Disease*, 2020;208(10:81-84. doi:10.1097/NMD.0000000000001089.

[65] Cook CCH, Hearing Voices, Demonic and Divine: Scientific and Theological Perspectives. Oxon (UK): Routledge; 2019. Chapter 1, Voice hearing in contemporary spiritual and religious context. Available from: https://www.ncbi.nlm.nih.gov/books/NBK540483/

[66] William Shakespeare, *Much Ado About Nothing*, Act Three, Scene Two.

tongue, buds are embedded in the fungiform papillae, while they are within the circumvallate papillae in the posterior regions. Taste-sensing signals are transferred from taste cell receptors in the taste buds through peripheral processes of unipolar nerve cells located in the genicular ganglion of the facial nerve, the inferior ganglions of the glossopharyngeal and vagus nerves[67]. The chemoreceptive events within the taste cells can detect different varieties of substance, there being five basic tastes, sweet, unami (essentially the flavor of glutamates), bitter, sour and salty. The taste receptors are generally considered to be G-protein-coupled receptors and channel-type receptors.

Several conditions affect the tongue, some of hereditary origin (e.g., macroglossia, or big tongue), of infectious origin (thrush, herpes), or cancer. It is the latter condition that is the most difficult to treat since it often involves resection of the tongue, known as glossectomy; this may also affect the floor of the mouth, the soft palate, oropharynx, and the mandible. Free-flap reconstruction of the tongue has been performed for several decades, with variable results, the technique having to consider qualities of speech, swallowing, and general quality of life[68].

There is little to report about the spiritual aspects of tongues; they are usually ignored in most cultures. Glossolalia, the so-called speaking with tongues of the Pentecostal Church is an important element of their spirituality, but there is much controversy about whether this is a divine language or just a phenomenon in emotionally charged people[69]. Sticking out your tongue is generally considered unacceptable behavior, although occasionally, as in Tibet, is used as a greeting. To the Maori people of New Zealand it is considered as a war chant, meant to be intimidating, hence its use in the hakka demonstration by the All Blacks rugby team before international matches;

Figure 2.28. The use of the tongue as an intimidating action in the All Blacks' Hakka.

[67] Fabian TK, Beck A, Fejerdy P, *et al,* Molecular mechanisms of taste recognition: Considerations about the role of saliva. *International Journal of Molecular Sciences*, 2015;16:5945-5974. doi:10.3390/ijms16035945.
[68] Vincent A, Kohlert S, Lee TS *et al*, Free-flap reconstruction of the tongue. *Seminars in Plastic Surgery,* 2019;33:38-45. doi:10.1055/s-0039-1677789.
[69] Amanze JN, Shanduka T, Glossolalia: Divine Speech or man-made language? A psychological analysis of the gift of speaking in tongues in the Pentecostal Churches in Botswana, *Studia Historiae Ecclesiastica*e, 2015, 41:1. doi:10.17159/2412-4265/2015/v41nn1a2.

2.2.1.8.4 Nose

Visually the nose does not have much attraction, as visible and as marked as the ears. In a poetic re-write of a few lines of Richard Llewellyn's memorable story "*How Green was my Valley'*, the young Huw Morgan was to say:

> *To rub noses*
> *Like Eskimos and Maori*
> *Looks silly now, isn't it*
> *But there is nothing to say*
> *About the taste of the nose*
> *The grisly triangle protruding from the face*
> *A nuisance in winter*
> *Just a friend in garden and kitchen*[70].

The nose serves as an entrance to the respiratory tract and contains the olfactory organ. It has two cavities, separated by the cartilaginous septum. The external openings are known as nostrils. The nasal cavity has a complex shape. The forward section, within and above each nostril, is the vestibule, behind which and along each outer wall are three elevations, running from front to rear. Each elevation, called a nasal concha or turbinate, is placed over an air passage; beside and above the uppermost concha is the olfactory region, the rest of the cavity being the respiratory region. This respiratory area is lined with a moist mucous membrane that has with fine hairlike projections, the cilia, which collect debris. Mucus from cells in the membrane wall also helps to trap particles and bacteria. Sinus cavities are present in the bone on either side of the nose. In the olfactory region, most of the lining is mucous membrane but a small segment of the lining contains nerve cells that constitute the sensory organ. Fibrous dendrites project from the nerve cells into the nasal cavity and are covered only by a thin layer of moisture. The moisture dissolves microscopic particles that the air has carried into the nose, stimulating the olfactory nerve cells chemically.

The olfactory system in humans is quite weak compared to that of many animals, but it is very precise[71], being able to detect and discriminate millions of chemical species, described as odorants, when in miniscule quantities. The odorants bind to specialized olfactory receptors, encoded by a large group of olfactory receptor genes belonging to the G-protein coupled family. Chemical information encoded in the odorants is converted into neuronal action potentials, which causes depolarization of olfactory sensory neurons. These signals are relayed to different parts of the brain for processing via the olfactory bulb.

The loss of smell is called anosmia. This is one of the key early symptoms of COVID-19 infections, and more papers have been published about it in the years 2020 / 2021 than in all previous years. Chronic anosmia is not very common, and often associated with chronic inflammatory diseases, trauma and toxic exposure. Many remedies have been suggested over the years, but with little success; most chemosensory disorders are irreversible, while some spontaneously remit without any clinical intervention[72].

Apart from anosmia, there are injuries and congenital deformities to consider. Since the nose is so prominent, much effort has been devoted to surgical procedures to provide more acceptable appearances.

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[70] David Williams, '*How Strange and Not-Strange is a Kiss'* Unpublished poem, 2019, Adapted from Richard Llewellyn*, "How Green was my Valley'* Michael Joseph, London, 1939.

[71] Sharma A, Kuar R, Aier I, *et al*, Sense of smell: Structural, functional, mechanistic advancements and challenges in human olfactory research, *Current Neuropharmacology*, 2019;17:891-911. doi:10.2074/1570159X17666181206095626.

[72] Doty RL, treatments for smell and taste disorders: A critical review. In *Handbook of Clinical Neurology,* 2019, (3rd series), Vol 164, Chapter 25. doi:10.1016/B978-0-444-63855-7.00025-3.

At one end of the range are those interventions aimed at straightening a deviated nasal septum, one of the most common surgical procedures in otorhinolaryngology (the clinical features of ear, nose, and throat medicine)[73]. This procedure is known as septoplasty, the benefits of which in terms of quality of life are difficult to assess. At the other end of the spectrum, serious cases of congenital absence of the nose (arrhinia)[74], trauma[75] and nasal cancer[76] are all treatable with some success that involves reconstruction, either with flaps or prostheses, or both.

One final point, as discussed by Sarafoleanu *et al,* the olfactory sense has a considerable capacity to modulate human behavior, playing a determinant role in the evolution of human habitat and social interactions; odor could be considered to be essential in defining human inner ego, tracing limits between professions, races and diseases[77].

2.2.1.8.5 The skin

The skin has three significant layers, the epidermis, the dermis, and the fat layer. The stratified squamous epithelium of the former primarily consists of keratinocytes in progressive stages of differentiation. Keratinocytes produce keratin, the major structural protein. The epidermis is avascular, and it is dependent on the underlying dermis for transport of delivery and waste products. The epidermis is a barrier to the external environment, preventing penetration by irritants and maintaining homeostasis. The epidermis is itself composed of several layers, either four or five (in the thickest areas including the palms of the hand and soles of the feet). The layers are, from the outside, the stratum corneum, the stratum lucidum (only in thick skin), the stratum granulosum, the stratum spinosum and the stratum basale. Although keratinocytes comprise around 95% of the epidermal cell population, there are other cells including melanocytes, Langerhans cells and Merkel cells. Keratinocytes are formed by division in the stratum basale and move through the stratum spinosum and stratum granulosum, where they differentiate to form a rigid structure of keratin, microfilaments, and microtubules. The stratum corneum has layers of flattened dead cells (corneocytes) which are shed from the skin in a desquamation process that takes about 28 days. Corneocytes attract and hold water, causing them to swell, keeping the stratum corneum pliable and elastic.

The dermis forms the inner layer of the skin and is much thicker than the epidermis. The network of interlacing connective tissue, which is its major component, is made up of collagen, in the main, with some elastin. Scattered within the dermis are several specialized cells (mast cells and fibroblasts) and structures (blood vessels, lymphatics, sweat glands and nerves). The dermis has two layers, the more superficial papillary dermis and the deeper reticular dermis. The papillary dermis consists of loose connective tissue containing capillaries, elastic fibers and collagen. The reticular dermis has a thicker layer of dense connective tissue containing larger blood vessels, closely interlaced elastic fibers and thicker bundles of collagen, also with fibroblasts, mast cells, nerve endings, lymphatics and epidermal appendages. Surrounding these structures is a viscous gel that allows the passage of nutrients, hormones and waste products, provides lubrication between the collagen and elastic networks, and gives bulk, allowing the dermis to act as a shock absorber. There is a complex network of blood vessels in the dermis

[73] Sommer F and Hoffmann T, Septoplasty – a surgical or political challenge. *Lancet,* 2019, 396. doi:10.1016/50140-673(19)31241-3.

[74] Jung JW, Ha, D-H, Kim BY, *et al,* Nasal reconstruction using a customized three-dimensional printed stent for congenital arhinia; three-year follow-up, *Laryngoscope*, 2019;129(3):582-585. doi:10.1002/lary.27335.

[75] Nuseir A, Hatamieh MM, Alnazzawi , *et al*, Direct 3D printing of flexible nasal prosthesis: Optimized digital workflow from scan to fit. *Journal of Prosthetics*, 2019;28(1):10-14. doi:10.1111/jopr.13001.

[76] Gallia, G.L., Reh, D.D., Salmasi, V. *et al.* Endonasal endoscopic resection of esthesioneuroblastoma: the Johns Hopkins Hospital experience and review of the literature. *Neurosurgery Review,* 2011, 34, 465–475. doi:10.1007/s10143-011-0329-2.

[77] Sarafoleanu C, Mella C, Georgescu *et al,* The importance of the olfactory sense in the human behavior and evolution, *Journal of Medicine and Life,* 2009;2(2):196-198. PMID 20108540.

which plays an important part in thermoregulation. The superficial plexus is made up of interconnecting arterioles and venules at the epidermal border suppling oxygen and nutrients to the cells while the deep plexus has more substantial vessels and is connected vertically to the superficial plexus. The lymphatic drainage of the skin is important, the main function being to conserve plasma proteins and scavenge antigenic foreign material and bacteria.

The hypodermis is the subcutaneous layer below the dermis, consisting mostly of fat. It provides structural support for the skin, insulating the body from cold and aids shock absorption.

Sensory receptors exist in all layers of the skin. Signals from the skin may be conveyed by physical change (mechanoreceptors), temperature (thermoreceptors), or pain (nociceptors). Mechanoreceptors respond to physical stimuli such as touch, pressure, vibration, and stretch. There are several different types of skin mechanoreceptors, including those around hair follicles, Pacinian corpuscles, Meissner corpuscles, Merkel complexes, Ruffini corpuscles, and C-fiber low threshold mechanoreceptors. Hair follicles can detect light touch; Meissner corpuscles in the dermal papillae detect indentation forces; Pacinian corpuscles in the deeper dermis detect vibration; Merkel complexes in the basal epidermis are responsive to texture; Ruffini corpuscles detect stretch; C-fibers detect light tactile sensations.

There are both warm and cold thermoreceptors. These receptors display a constant discharge to their specific temperatures, and when an experience of the opposite temperature occurs, there is a sudden ceasing of receptor discharge. Cold receptors mainly sense temperatures between 25 to 30ºC. Warm receptors respond to the range of 30 to 46ºC. Higher temperatures may result in the decreased firing of these receptors.

Nociceptors signal pain related to temperature, pressure, and chemicals. Nociceptors only signal when the body has reached a point of tissue damage. Inflammatory markers increase during damage, binding to receptors, and initiate pain signaling either externally or in the viscera, for example, with ion channel families that are present on nociceptive neurons. Different fibers relay pain information, fibers differ in their myelination and nerve diameter and thus speed of transmission. Painful temperatures mostly use C-fibers, while A-delta fibers are small and unmyelinated and are primarily involved in thermal and mechanosensitive pain.

There are many conditions that affect the skin, some minor in nature, some very serious; many are related to infectious processes, either bacterial, fungal or viral, while others may be related to cancer or autoimmunity. Examples include acne, atopic or contact dermatitis, eczema, herpes, leprosy, scleroderma, vitiligo, psoriasis and basal cell or squamous cell carcinoma. Most conditions can be treated pharmacologically, and few are considered as targets for reconstruction. Some conditions, such as diabetic foot ulcers, are caused by problems in the underlying tissues rather than by the skin *per se* and are considered elsewhere. Many cases of injuries to the human body involve the skin, and again such aspects will be considered in more appropriate places, for example if they involve open bone fractures.

The one condition of skin that does deserve attention here is that of a burn injury. In the USA alone there are as many as a half-million burn injuries each year, ten per cent of which require hospitalization for treatment. The burn depth and body surface affected area are the predictors of morbidity and mortality. Generally, burns are defined within three categories. First-degree burns, are superficial, affecting only the epidermis, with minimal damage. Second-degree burns penetrate the dermis and are described as of partial thickness. Burns that penetrate the dermo-epidermal junction create blisters that can only self-repair when the basal layer of keratinocytes is able to regenerate the layer. Deep partial-thickness burns reach the reticular dermis, affecting hair follicles and sweat and sebaceous glands; these may take 3 weeks to heal and are likely to leave a noticeable hypertrophic scar.

Third-degree, or full thickness, burns penetrate the dermic and hypodermic, destroying blood supply and nerves. These are slow to heal and make require extensive reconstruction, especially if they affect deeper

tissues, including adipose and muscle tissue. Burn debridement, or removal of devitalized tissue by hydrotherapy is a first step in treatment, and this will be followed by surgical debridement. Following this, the standard of care for treatment will involve autologous skin grafting, either full-thickness or split-thickness. A great deal of attention has been given in recent years to the use of so-called skin substitutes[78]. The use of these and all types of skin graft are covered later in the section on enabling technologies.

Both spiritual and religious practices, however they are defined, have a particular focus on skin, partly because it is one of the more obvious manifestations of a person, and partly because it provides a physical barrier to the outside environment and is readily visible and touchable to the self and others[79]. Skin, through visible changes in color and warmth, reflects emotional states, and appearances reflect and influence thoughts, and vice versa. Common metaphors relating to the skin, as in thin- or thick-skinned add to the spiritual core. Humans intentionally exaggerate or suppress their outward appearance through the use of clothing and skin markings and coverings, such as tattoos and make-up, and skin piercing. Visible skin disorders can have marked psychological and social effects, which are not necessarily present with internal disorders. Also, any reconstructive procedures that result in different skin appearances in different parts of the body, as they almost inevitably will, or which result in unsightly keloid scars, can have significant psychological effects[80].

2.2.2 Tissues and Tissue Systems

2.2.2.1 Nervous System

The brain was discussed in the previous section in the context of its definition as an organ. The distinction between the brain and the rest of the nervous system may seem a little arbitrary, especially as that system is normally considered to have two main parts, the central nervous system (CNS), which includes the brain and the spinal cord, and the peripheral nervous system, which comprises of all the nerves that branch from the spinal cord. The interface between the brain itself and the rest of the nervous system is found at the brainstem, that area at the base of the brain that lies between the deep structures of the cerebral hemispheres and the cervical spinal cord. It is divided into three parts: the midbrain (mesencephalon), the pons (metencephalon), and the medulla oblongata (myelencephalon); it has a critical role in the regulation of some involuntary actions, including breathing. Many cranial nerve nuclei are located in the brainstem, providing motor and sensory function to parts of the cranium, including the facial muscles, tongue and larynx, as well as responding to the senses of taste and hearing. It also has nuclei important for sympathetic and parasympathetic autonomic functions.

2.2.2.1.1 Basic terminology and structures

The nerve cell, or neuron, is the basic unit of the nervous system. The structure and function of neurons are discussed elsewhere, but it should be noted here that a neuron has a cell body, which includes its nucleus, and extensions called axons and dendrites. Almost all neurons have a single axon and multiple dendrites. Axons are long processes which conduct action potentials away from the cell body; bundles of axons constitute the anatomical structure that is commonly called a nerve. Dendrites receive chemical signals from the axon termini of other neurons. Connectivity between neurons is achieved through synapses, which are junctions between the end of axons and the tips of dendrites of other cells. It is here

[78] Tavakoli S and Klar AS, Bioengineered skin substitutes: Advances and future trends, *Applied Sciences,* 2021;11:1493. doi:10.3390/app11041493.
[79] Shenefelt PD and Shenefelt DA, Spiritual and religious aspects of skin and skin disorders, *Psychology Research and Behavior Management*, 2014;7:201-212. doi:10.2147/PRBM.S65578.
[80] Jeschke MG, van Baar ME, Choudhry MA, *et al,* Burn injury, *Nature Reviews: Disease Primers*, 2020;6:11. doi:10.1058/s41572-020-0145-5.

that neurotransmitters receive an electrical signal from an axon, which is converted into a chemical signal that is then passed to the neighboring dendrite and converted back into an electrical signal. Around most axons is a protein / polysaccharide insulating substance, myelin; this myelin sheath enhances the speed with which signals pass along the nerve. The myelin sheath is interrupted along the length of the axon by regions called the nodes of Ranvier, which are essential for the speed and timing of delivery of impulses from one neuron to another. Changes in size or function of these can jeopardize the effectiveness of a neuron and the onset of neurological disorders.

2.2.2.1.2 Spinal cord

The spinal cord extends from the brainstem, running from the level of the first cervical vertebra to that of the twelfth thoracic vertebra. The spinal cord and the surrounding vertebral column are divided into cervical, thoracic, lumbar, sacral, and coccygeal regions. The cervical region gives rise to eight cervical nerves (C1—C8), the thoracic to twelve thoracic nerves (T1—T12), the lumbar to five lumbar nerves (L1—L5), the sacral to five sacral nerves (S1—S5), and the coccygeal a single coccygeal nerve. These nerves leave the vertebral column through the intervertebral foramina of the vertebral body. Sensory information carried by the afferent axons of the spinal nerves enters the cord *via* the dorsal roots, and motor commands carried by the efferent axons leave the cord *via* the ventral roots. Where the dorsal and ventral roots join, sensory and motor axons travel together in the segmental spinal nerves.

Two regions of the cord are enlarged to accommodate the nerve cells and connections needed to process information related to the upper and lower limbs. The part that corresponds to the arms is called the cervical enlargement and includes spinal segments C5—T1, that corresponding to the legs being the lumbar enlargement which includes spinal segments L2—S3. Because the spinal cord is considerably shorter than the vertebral column, lumbar and sacral nerves run for some distance in the vertebral canal before emerging, thus forming a collection of nerve roots known as the cauda equina.

The spinal cord consists of gray and white matter. The gray matter contains the cell bodies of motor and sensory neurons, while the white matter has interconnecting fiber tracts that are primarily myelinated sensory and motor axons. Following longitudinally through the center of the cord is the central canal, which is continuous with the ventricles of the brain and contains CSF. The white matter is organized into tracts, both ascending and descending. The former carry information from sensory receptors to high levels of the CNS while the descending tracts transit information towards the periphery. Within the gray matter there is a laminar distribution of neurons that have a variety of functions. Also within the spinal cord are astrocytes, which are cells that secrete neurotrophic factors, and oligodendrocytes, which produce myelin.

Rather like the protective layers surrounding the brain, the spinal cord is surrounded by connective tissue layers, the meninges. Directly lining the cord is the pia mater. Then there is the arachnoid mater, the space between these two layers being the sub-arachnoid space, which also contains CSF.

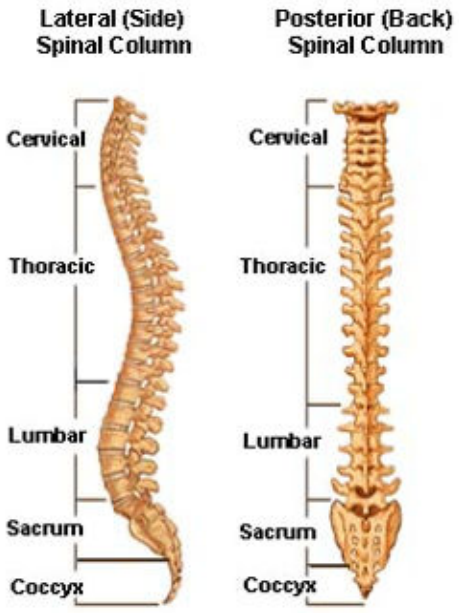

Figure 2.29. Lateral and posterior views of the human spinal column.

The top layer is the dura mater, with the epidural space separating the meninges from the vertebral column. Spinal nerves exit the cord as rootlets, which join to form the nerve roots. The anterior nerve roots consist of motor neurons, each of which have one axon, two dendrites and some collateral branches; these control skeletal muscles and the autonomic nerve system. The posterior nerve roots contain sensory fibers and dorsal root ganglia. Anterior and posterior nerve roots converge into spinal nerves. Outside the vertebral column, the spinal nerves divide into several branches, including the dorsal ramus which contains nerves carrying visceral motor, somatic motor, and sensory information to and from the skin and muscles of the back and the ventral ramus, which contains nerves that carry visceral motor, somatic motor, and sensory information to and from the ventrolateral body surface, structures in the body wall, and the limbs. Some ventral rami merge to form a nerve plexus, a network of interconnecting nerves, which are carried together to target locations, including the cervical, brachial, lumbar, and sacral plexuses. The meningeal branches exit the spinal nerve and re-enter the intervertebral foramen to serve the ligaments, dura, blood vessels, intervertebral discs, facet joints, and periosteum of the vertebrae.

Two major conditions affect the spinal cord. The first is of developmental origin which occurs when neural tubes fail to close. When this failure occurs in distal positions the result is a myelomeningocele, referred to as open spinal bifida. The second involves injury to the spinal cord. Immediately after injury, cells of the immune system migrate to the site, causing damage or death to some neurons. The death of oligodendrocytes causes axons to lose their myelination, impairing the conduction of action potentials or rendering the remaining connections useless. If many axons are severed, communication between the brain and muscles and between the body's sensory systems and the brain is compromised. Usually, the area of tissue damage is cleared away, but a fluid-filled cavity surrounded by a glial scar is left behind. which acts as a barrier to the reconnection of the two sides of the damaged cord. The cord area may have ‘healed’ but will often be functionally useless; hence the profound nature of spinal cord injury and the significant need for technologies that can overcome this natural but ineffective repair process and, instead, reconstruct a proper functioning cord.

2.2.2.1.3 Peripheral nervous system

The peripheral nervous system is divided into two parts, the somatic and the autonomic nervous systems. The somatic system is responsible for carrying sensory and voluntary motor information to and from the central nervous system. It contains two major types of neurons: motor neurons carry information from the brain and spinal cord to muscle fibers throughout the body and sensory neurons carry information from the nerves to the brain and spinal cord. The autonomic system is that part which is responsible for regulating involuntary body functions, such as blood flow, heartbeat, digestion, and breathing. This system has two branches: the parasympathetic system helps maintain normal body functions and conserve physical resources, slowing the heart rate and breathing, and reducing blood flow to muscles, as necessary, allowing the body to maintain normal resting state. The sympathetic system prepares the body to expend energy in response to environmental threats, for example by accelerating heart rate, increasing breathing rate, boosting blood flow to muscles, and activating sweat secretion.

The somatic peripheral nervous system includes the sensory and somatosensory systems, the latter referring to sensations, for example pain and heat, which can occur anywhere in the body. There are twelve cranial nerves, ten of which originate from the brainstem and control the functions of the anatomic structures of the head. Nerves derived from the spinal cord control the functions of the rest of the body; there are 31 pairs of these nerves, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal. The first 4 cervical spinal nerves, C1 through C4 serve the neck and back of head. Examples are the suboccipital nerve, which provides motor innervation to muscles at the base of the skull and the greater occipital nerve that provides sensation to the back of the head. The phrenic nerve is particularly important, arising from C3 to C5 which controls thoracic diaphragm and, in consequence, breathing. Cervical spinal nerves C5 to C8, and the first thoracic spinal nerve, T1, combine to form the brachial plexus that serves the upper-limb and upper back.

The autonomic nervous system is always activated but is either in the sympathetic or parasympathetic state. Depending on the situation, one state can overshadow the other, resulting in a release of different kinds of neurotransmitter. In sympathetic mode, norepinephrine and epinephrine are released in situations that demand increased heart rate and blood flow in muscle. Through the release of acetylcholine**,** the parasympathetic system can dominate the body, with increases in salivation and digestion patterns, while heart rate and other sympathetic response decrease. Unlike the sympathetic system, there is some voluntary control in the parasympathetic system, for example with urination and defecation.

Disorders of the peripheral nerve system can arise from damage to or dysfunction of the cell body, myelin sheath, axons, or neuromuscular junctions. They may be of genetic origin, or acquired, for example, trauma or toxic, metabolic, infectious, or inflammatory conditions; these are referred to as peripheral neuropathies and they may affect one nerve or several discrete nerves, multiple nerves diffusely, a plexus or a nerve root. Sensory and motor cell bodies are in different locations so that a nerve cell body disorder usually affects either component but rarely both. Damage to the myelin sheath, referred to as demyelination, slows nerve conduction and causes large-fiber sensory dysfunction (such as tingling sensations) with motor weakness and diminished reflexes. Some peripheral nerves are vulnerable to vascular disorders such as vasculitis and ischemia, which can result in small-fiber sensory dysfunction, motor weakness proportional to atrophy, and less severe reflex abnormalities than in other nerve disorders. Toxic-metabolic or genetic disorders usually begin symmetrically. Immune-mediated processes may be symmetric but can be asymmetric in rapidly evolving processes. Damage to the myelin sheath can often be repaired by surviving Schwann cells in around 10 weeks. After axonal damage, the fiber regrows within the Schwann cell tube at about 1 mm/day, but regrowth may be misdirected, causing aberrant innervation. Regeneration is impossible when the cell body dies and is unlikely when the axon is completely lost.

2.2.2.2 Muscles, Tendons, and Ligaments

The musculoskeletal system orchestrates locomotion while at the same time supporting the body and protecting internal organs. The system consists of bones, cartilage, tendons, ligaments, muscle, and connective tissue, as well as the joints, which are effectively sub-systems that involve several of these types of tissue and, in many cases, specialized fluids that facilitate articulation. It is convenient here to separate the soft tissues of muscles, tendons, and ligaments, which are dealt with in this section, and the bones and joints covered afterwards. It is important to note that some of these terms are generic, there being muscles and ligaments in the body but outside of the musculoskeletal system. These will be discussed in relevant places and should not cause confusion.

2.2.2.2.1 Skeletal muscles

As implied above there are several types of muscle in the human body; skeletal muscle is discussed here, the other two being cardiac and smooth muscle. The human body contains over 600 individual skeletal muscles, many being located in pairs on either side of the body, for example in the arms and legs. The muscles often work in groups to achieve coordinated actions. Some of the major muscle groups are the pectoralis major and latissimus dorsi within the upper torso, gluteus maximus and iliopsoas in the lower torso, biceps brachii in the arms and quadriceps femoris in the legs. These names have familiar shortened versions when used in physical therapies and sports activities, such as biceps, glutes, pecs and psoas.

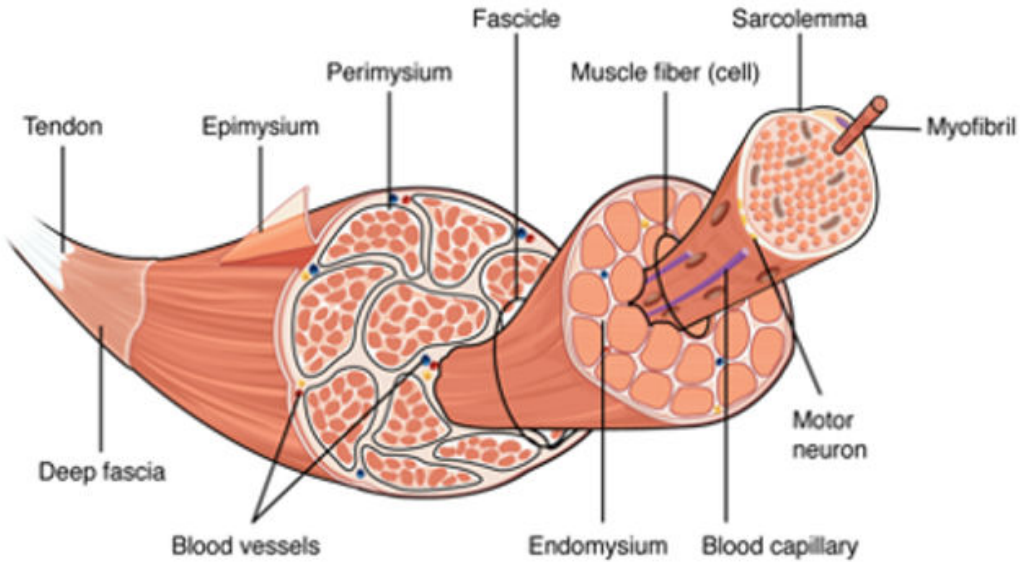

Figure 2.30. Structure of skeletal muscle.

Skeletal muscles consist of large numbers of muscle fibers, which are elongated cells, wrapped together by sheaths of connective tissue; most of these cells are multinucleated so that they are fully functional over their entire length. Individual bundles of fibers are known as fasciculi. The outermost sheath surrounding the entire muscle is the epimysium, while that covering each fasciculus is the perimysium, and the innermost sheath surrounding individual fibers is the endomysium. Each muscle fiber is comprised of several myofibrils which themselves contain myofilaments. Together, the myofibrils are arranged in a striated pattern (with a distinctive appearance of fine red and white lines), forming structural subunits known as sarcomeres which are the fundamental contractile unit of a skeletal muscle. There are

two significant types of myofilaments, actin and myosin, filaments which again are arranged with a distinctive banding form.

The neuromuscular junction is a synaptic connection between the terminal end of a motor nerve and a muscle, acting as the site for the transmission of action potential from nerve to muscle. It provides an excellent example of the criticality of linkages in the integrated system that is the human body. The neuromuscular junctions have three main parts, a presynaptic nerve terminal, a postsynaptic motor endplate, and a synaptic cleft that is placed between them. The nerve terminal is a myelinated motor neuron that loses its myelin sheath within the target muscle, forming a complex of 100-200 branching nerve endings, or terminals. The nerve terminal membrane has areas, the active zones, with thicker membranes, which involve a family of SNAP proteins and rows of voltage-gated calcium channels. It also has potassium channels and contains mitochondria and synaptic vesicles, the latter storing around the neurotransmitter acetylcholine. The motor endplate is the thickened portion of the muscle plasma membrane that is folded to form junctional folds, into which the terminal nerve endings fit. These folds have receptors that are acetylcholine - gated ion channels, where binding to these receptors opens the channels to allow influx of sodium ions from the extracellular fluid into the muscle membrane, creating an endplate potential that generates and transmits action potentials to the muscle membrane.

The primary artery supplying blood to the muscle follows the longitudinal axis of the muscle fiber and gives off feed arteries in a perpendicular direction, proceeding towards the external connective tissue sheath. The feed artery branches into primary arterioles, further branching giving transverse arterioles and then terminal arterioles which perfuse the capillaries that are present. Lymph capillaries originate in skeletal muscle in the microvascular unit within the endomysium near the main capillary bed and drain the tissue fluid. The neuronal innervation consists of sensory and motor nerve fibers, together with neuromuscular junctions. The cell bodies of the neurons have large axons which divide into multiple smaller branches to innervate multiple muscle fibers. The motor nerve terminal has abundant mitochondria, endoplasmic reticulum and numerous synaptic vesicles containing acetylcholine.

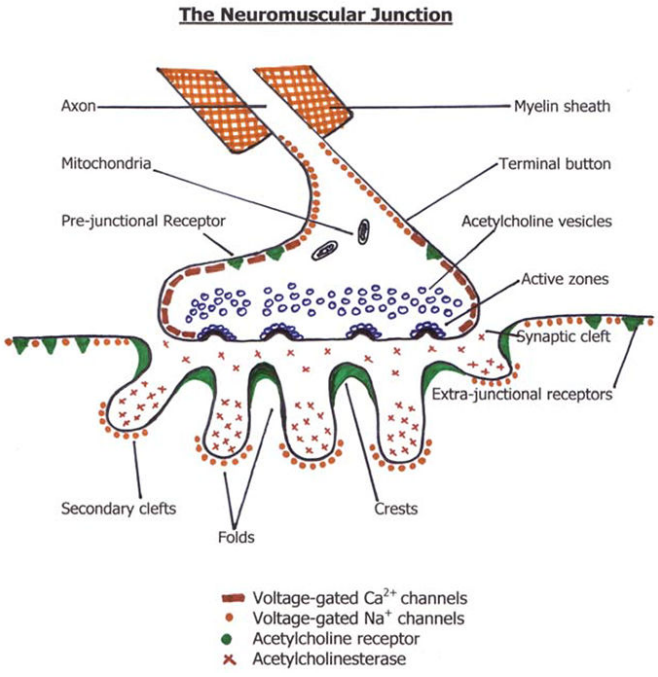

Figure 2.31. The neuromuscular junction.

The primary functions of the skeletal muscle are delivered *via* intrinsic excitation-contraction coupling processes. The muscle is attached to the bone through tendons, so that the contraction of the muscle leads to specific movements. Skeletal muscle contraction begins first at the neuromuscular junction. Excitation-contraction coupling refers to the process that converts the action potentials in the muscle fibers into muscle fiber contraction. The action potentials at the cell membrane surrounding the myofibrils travel into T-tubules, which contain dihydropyridine receptors that are adjacent to the terminal cisternae of the sarcoplasmic reticulum of the muscle fiber. When T-tubules become depolarized, these receptors mechanically interact with the ryanodine receptors on the sarcoplasmic reticulum, causing release of calcium, which binds to troponin, which in turn causes displacement of tropomyosin from the myosin-binding sites on F-actin. The thick and thin filaments slide past one another to generate a muscle contraction. At the beginning of the cycle, when myosin is tightly bound to actin, no adenosine triphosphate is bound to myosin, in a transient state in contracting muscle. Next, ATP binds to the myosin head, decreasing its affinity for actin. Consequently, myosin dissociates from actin myosin then binds to a new site on the actin, creating a power stroke that pulls the actin filaments. After contraction, muscle relaxation occurs when calcium reaccumulates in the sarcoplasmic reticulum.

Since skeletal muscle constitutes around 40% of the body volume, it must be considered as a primary target for diseases and other conditions, including trauma. It is possible to consider these conditions as either of primary or secondary nature but since some have origins in both nervous and muscular systems, the distinction is not necessarily helpful. Among those that are clearly primary muscle diseases, muscular dystrophy is a group of inherited diseases which cause muscle loss and weakness. The conditions may occur in infancy or childhood, but often this does not happen until middle age. Symptoms are specific to the type of disease and vary with the muscle groups and individuals. The conditions are progressive, most of those affected losing the ability to walk. Symptoms can be treated, and complications can be mitigated or prevented. There are no cures so that muscular dystrophy is of major interest for reconstructive technologies. Rhabdomyolysis is a muscle disease that involves the breakdown of skeletal muscle, which causes myoglobin release into the bloodstream, which can lead to kidney damage. Causes of rhabdomyolysis include trauma, infection and inflammation, medications, toxins, and genetic factors. Kidney damage from rhabdomyolysis may not be reversible, again indicating a role for organ replacement.

Among neuromuscular diseases that have a neurological basis, but which severely affect skeletal muscles, is myasthenia gravis, an autoimmune disorder that results from impaired communication between nerve cells and muscles. This impairment reduces muscle contractions and muscle weakness eventually occurs. Again, there is no cure, but treatments are more likely to be based on the immune system. Amyotrophic lateral sclerosis, also called Lou Gehrig's disease, is a group of rare neurological diseases of the nerve cells in the brain and spinal cord, which affects the muscles responsible for voluntary movement. There is no cure; symptoms will get worse with time and typical life expectancy is only a few years after diagnosis. Treatments are palliative and supportive, again with much hope being placed on future regenerative technologies. Sarcopenia is a muscle disease common in older adults, which causes muscle mass and strength loss. It is likely that more than 50% of adults in developed countries show symptoms of sarcopenia, risk factors including poor nutrition, chronic inflammatory diseases, and reduced hormone levels. Physical therapy, improved diets, and medications can improve the condition, but reconstructive procedures seem irrelevant at this time.

The multiple interconnected movements within the complex musculoskeletal system lead to many possible areas that can increase the risk of muscle injury. Many underlying factors, including fatigue, weakness, impaired nutritional status, age, gender, illness, drugs, and previous injury may be associated with injury. Muscle injuries are classified according to the type, locations, and severity of tissue damage; they may also be grouped as primary or secondary injuries. Primary injuries are the most common, the pathophysiology of each being unique in its mechanism; the three principal types are

contusions, lacerations, and sprains. Contusion injuries are the result of blunt force directed into the muscle, being characterized by hematoma formation and only minor superficial disruption of tissue. In lacerations, the insult is localized, sharp dissection of tissue, the damage often being more noticeable than with blunt injuries. The most common primary muscle injuries are strains. Severe strains have full or nearly full disruption of the muscle and are associated with significant impairment of function. The reality, nevertheless, is that the majority of these primary injuries, mostly arising from sports, travel and domestic activities, are treatable and do not require reconstructive technologies; the skeletal muscle has a high endogenous capacity for repair through the presence of satellite cells and the cell-signaling cascade that they release on such injury. A major example of secondary injury is compartment syndrome, which is characterized by pathologically increased tissue pressure within a confined space. Severe blunt trauma has the potential to cause fluid accumulation either because of the local inflammatory response to the trauma or, if the trauma is severe enough, by compromised vasculature within the muscle. Both processes result in an increase in pressure within a confined space, leading to arteriole compression and an ischemic insult, considered an acute surgical emergency.

Unfortunately, the self-repair process is not so effective with severe injury, when the signaling cascade mentioned above is overwhelmed, which results in a persistent pro-inflammatory environment and the development of fibrosis or fat instead of functional muscle. Such conditions, referred to as volumetric muscle loss, especially arise in military-related trauma[81]. Reconstruction is very difficult, as will be discussed later.

2.2.2.2.2 Tendons

Tendons are the structures that connect muscles to bones[82]. They have varied shapes and sizes; extensor tendons are more flattened than the rounder flexor tendons, this being determined by the nature of the bony surfaces to which they relate. The ECM of tendons consists of collagens and proteoglycans, the former establishing the tendon strength and the latter, its viscoelasticity. The hierarchical structure of tendons is determined by the complexity of the mechanical demands. This consists of polypeptide chains of the collagen forming a densely packed helical tropocollagen molecule, five of which constitute a microfibril, which aggregate to form fibrils, which are grouped into fibers, then into fiber bundles and then into fascicles. At various levels, a helical architecture is formed, giving considerable flexibility. The fascicles are able to slide independently against each other, allowing them to transmit tension, notwithstanding the changing angles of a joint. Tenocytes are the specialized types of fibroblast that dominate the cellular components of tendons, being arranged on longitudinal rows close to the collagen fibrils. Tenocytes can modulate their activity according to mechanical load, and the properties of tendons considerable reflect the exercise regime applied to the musculoskeletal system.

Tendons are white in appearance; however, they are vascularized, although nothing like to the extent of the muscles to which they are attached. Vessels run longitudinal and are usually small and thin walled. Nerves are mostly derived from within the muscle. Tendons have satellite structures that facilitate sliding of the tendon around anatomical structures and prevent the tendon from losing its course of action during muscle contraction. The fibrous sheaths represent the sliding channels of the tendons, while the synovial sheaths facilitate sliding of the tendon inside the fibrous sheath.

Tendon disorders include ruptures and overuse injuries, although they also appear among the sedentary population with limited physical activity. Tendinopathies are disorders accompanied by inflammation and pain, whereas tendinosis and ruptures are caused by intertendinous degeneration. The prevalence of

[81] Smoak MM and Mikos AG, Advances in biomaterials for skeletal muscle engineering and obstacles still to overcome. *Materials Today Bio*, 2020;7:100069. doi:10.1016/j.mybio.2020.100069.
[82] Benjamin M, Kaiser E, Milz S, Structure-function relationships in tendon: A review. *Journal of Anatomy,* 2008:212:211-226. Doi:10.1111/j.1469-7580.2008.00864.x

Achilles tendinopathies and ruptures has risen in recent years due to an increase in the elderly population and a higher participation in excessive physical activities. Tendons possess the capability to heal by a repair process controlled by tendon cells and their surrounding ECM. Immediately after tendon injury, scar formation and tissue repair are initiated. The initial inflammatory stage begins with the formation of a hematoma shortly after injury. Secreted angiogenic factors initiate the formation of a vascular network that is responsible for the stabilization of the injury site. The remodeling stage begins six to eight weeks after injury and takes around one to two years. The first sub-stage, consolidation, is characterized by a decrease in cellularity and matrix production, as the tissue becomes more fibrous through the replacement of collagen type III by collagen type I. Collagen fibers then start to organize along the longitudinal axis of the tendon, thereby restoring tendon stiffness and tensile strength. The healed tendon does not usually regain the mechanical properties of the uninjured tissue; the tendon thickens and stiffens to overcome the lower mechanical strength and the tendon quality, and its functional activity are reduced. Achilles tendon ruptures are common, and reconstruction often requires an augmentation procedure such as a tendon transferor or tendon graft. Transfer of the tendon of the flexor hallucis longus is popular but is technically challenging and the great toe flexor strength is permanently impaired after the procedure. The transfer of peroneus brevis tendon usually leaves a decrease in eversion strength of the affected ankle. The gracilis tendon has been used for reconstruction of chronic tears of the Achilles tendon[83].

2.2.2.2.3 The story of Achilles Heel

It is widely recognized that the vulnerability of the tendon in the healing of the human body is reflected in the story / mythology surrounding the Greek warrior Achilles, this story being recounted by Homer in his Iliad. The father of Achilles was Peleus, the mortal king of the Myrmidons, a legendary fearless people, his mother being Thetis, a Nereid.

Thetis was concerned about her son's mortality and did everything she could to make him immortal: at one critical point, she immersed him in the River Styx, in order to confer the invulnerability of the gods. However, she gripped him tightly by the foot so that the water never touched his heel, which remained vulnerable. The Trojan War began when Zeus decided to reduce Earth's mortal population by arranging a war between the Greeks and the Trojans. At Achilles' parents' wedding banquet, Zeus invited the prince of Troy, named Paris, to judge the beauty of goddesses Hera, Athena, and Aphrodite. Aphrodite's was the most alluring and promised to give Paris the most beautiful wife. Unfortunately, the wife in question was Helen, the daughter of Zeus, who was already married to Menelaus, the king of Sparta. At Aphrodite's urging, Paris went to Sparta, won Helen's heart and took her back to Troy. Menelaus assembled an army of Greece's greatest warriors, including Achilles, and set off to get his wife back; this war lasted for ten years. Achilles met with great, undefeated, success but the war itself had reached a stalemate.

Because of internecine quarreling between the leading soldiers, which included Agamemnon, brother of Menelaus, the god Apollo punished the Greek armies by sending a plague to kill the soldiers. As his ranks diminished, Agamemnon had to agree to some Trojan demands, including the return of Achilles' wife, the Trojan princess Breseis. Achilles did as his commander asked and relinquished his bride but refused to come out to fight. The tide began to turn in favor of the Trojans. Eventually, Achilles' best friend, Patroclus, was able to produce a compromise: Achilles would let Patroclus use his powerful armor as a disguise so that the Trojans thought Achilles had returned to battle and would retreat in fear. Apollo intervened and helped the Trojan prince Hector to kill Patroclus. Achilles chased Hector back to Troy, and stabbed him in the throat, killing him. Achilles returned to Troy after Hector's funeral to exact further revenge for Patroclus' death. However, Paris ambushed Achilles as he entered Troy. He shot his enemy

[83] Maffulli N, Spiezia F, Testa V, *et al,* Free gracilis tendon graft for reconstruction of chronic tears of the Achilles tendon. *The Journal of Bone & Joint Surgery*; 2012;94:906-910. doi: 10.2106/JBJS.K.00869.

with an arrow, which Apollo guided to his vulnerable heel, which his mother's hand had protected from the waters of the Styx.

Figure 2.32. Achilles being immersed in the River Styx with Thetis holding him by his heel. Statue in The Walker Art Gallery, Liverpool.

There aren't many parts of the human body that are associated with such mythology. The Iliad is worth reading (at least in parts) to get a better picture of this ancient Greek landscape. It starts:

> *Rage – Goodness, sing the rage of Peleus; son Achilles,*
> *murderous, doomed, that cost the Achaeans countless losses,*
> *hurling down to the House of Death so many sturdy souls,*
> *great fighters souls, but made their bodies carrion*
> *feasts for the dogs and birds,*
> *and the will of Zeus was moving toward its end.*
> *Begin, Muse, when the two first broke and clashed.*
> *Agamemnon lord of men and brilliant Achilles.*
>
> **From Homer's Iliad**[84]

2.2.2.2.4 Ligaments

Ligaments are tough, fibrous connective tissue components that connect adjacent bones and provides stability to joints. Damage to ligaments is a very common feature of many sporting injuries and some of the more serious sites of such injuries, such as the cruciate ligaments in the knees and collateral injuries in elbows, are in common lay usage. There are nearly one thousand ligaments in the human body, which vary in size, shape, orientation, and location. Ligaments consists of dense bundles of collagenous fibers,

[84] Homer, *The Iliad,* Translated by Richard Lattimore, University of Chicago Press, 1961, ISBN 0226469409.

which are surrounded by the gel-like ground substance. The bundles of collagen attach to an outer covering of bones, the periosteum, where there is usually an additional lubricating synovial membrane, and pouch. The point of attachment is called an insertion. With most ligaments, the outer layer, the epiligament, is quite vascular; nerves tend to follow the blood vessels and are greater in number near the insertions. There are relatively few cells in ligaments, those present largely being fibroblasts.

There are five major shoulder ligaments all contained within the glenohumeral and acromioclavicular joint spaces. These are the superior, middle and inferior glenohumeral ligaments, which connect the glenoid to the humerus, and the acromioclavicular and coracoclavicular ligaments, which connect the upper part of the shoulder blade to the clavicle. Together, these ligaments give three degrees of freedom, allowing the upper arm to glide in multiple directions, but also making it more prone to injury. There are 7 ligaments supporting the spine: the ligamentum flavum is located between vertebrae, the facet capsular ligament at the capsular insertion points, the interspinous ligament, which is between the spinous processes, the supraspinous ligament which is above and at the side of each vertebra, the intertransverse ligament, located in between the long sides of vertebra, and the posterior and anterior longitudinal ligaments, which run along the side and front of the spine respectively. The latter two are the major contributors to spinal stability; injury to the posterior longitudinal ligament may result in disc herniation.

Damaged ligaments can heal, but a great deal depends on the extent and type of injury, the nature of any gaps in ligament tissue that result from the injury and the location. As with muscles and tendons, the healing process takes place *via* inflammation, repair and remodeling. Serious injuries to movement-critical ligaments may take many months to heal, and the final result may have functional deficits in view of the inferior properties of remaining scar tissue, especially with poorer strength, stiffness and energy-absorption characteristics.

2.2.2.3 Bones, Cartilage and Joints

In this section I deal with the bones and cartilage, which together comprise most joints of the body.

2.2.2.3.1 Bones

Bones are arranged within the skeletal system in two parts, the axial skeleton, arranged along the axis of the body, including the skull, vertebral column and ribcage, and the appendicular skeleton which consists of appendages outside of the axis, including the upper and lower limbs, and pelvic and shoulder girdles. Within these two systems are long, short, flat, irregular, and sesamoid bones. Long bones, which include the tibia, humerus, radius and femur, consist of the shaft, or diaphysis, with epiphyses at both ends, which join at the metaphysis. Short bones are typically cube-shaped and include bones in hands and feet. Most flat bones are located in the axial skeleton, including the ribs. Irregular bones primarily include the vertebra. Sesamoid bones are typified by the patella, into which tendons are inserted.

The two principal functions of bones are mechanical, with respect to movement, and protection. As seen in these sections on the musculoskeletal system, bones are attached to muscles, tendons and ligaments that provide a framework within which the energy delivered by muscle contractions is coordinated through to bones that act as levers, producing a wide range of movements. By virtue of their strength and toughness, bones are able to protect soft organs, for example the ribcage containing the heart and lungs, the cranium and vertebral column protecting the brain and spinal cord, and the pelvis containing the urogenital organs.

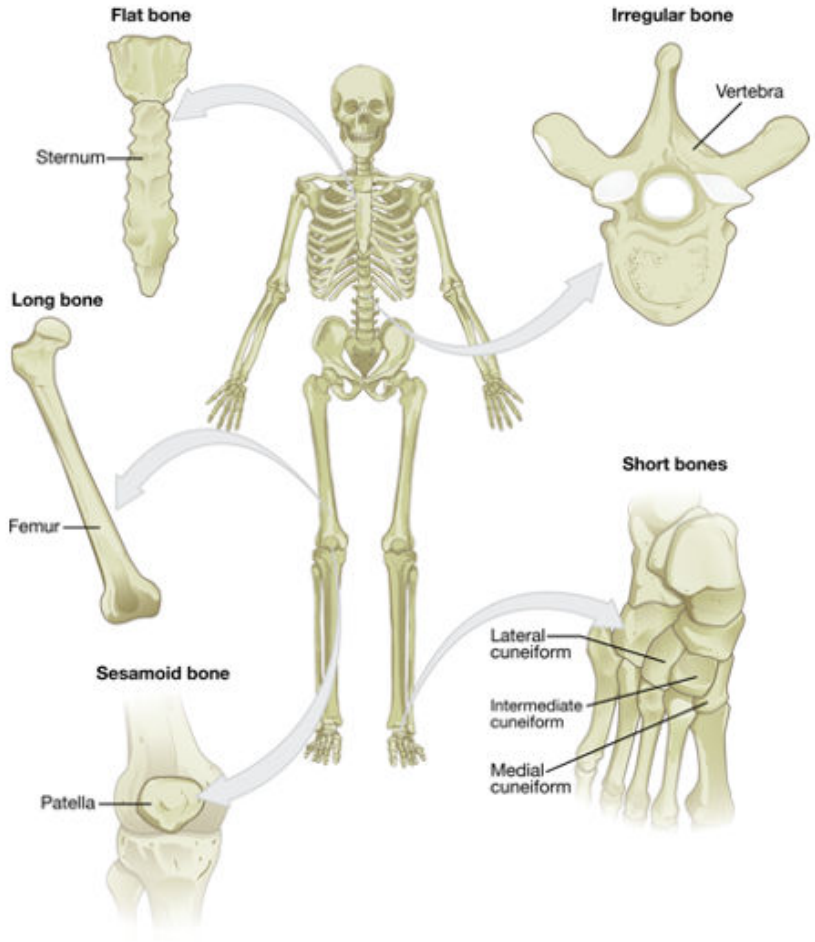

Figure 2.33. Types of bone in the human skeleton.

In addition, the bones act as reservoirs for minerals, not just the 99% of the body's calcium, but 85% of the phosphate and 50% of the magnesium. These minerals are released when necessary, levels being regulated by hormones, such as parathyroid hormone. Moreover, the bone marrow is responsible for the production of blood cells from haemopoietic stem cells. Bone is a complex composite material, consisting of an organic matrix and mineral phases distributed in this matrix. The organic phase is approximately 90% type-I collagen fibers, with 10% other molecules, such as glycoprotein, osteocalcin, and proteoglycans. The mineral phase is predominantly hydroxyapatite, a calcium phosphate, $Ca_{10}(PO_4)_6(OH)_2$, which is present as plates or needles, 40 to 60 nm long, 20 nm wide, and 1.5 to 5 nm thick. By volume, bone consists of about 40% hydroxyapatite, 35% organic matrix, the remainder largely being water.

The structure of bone is not uniform; there are two main types of bone, distinguished by their macroscopic and microscopic structures. Cortical bone, also referred to as compact bone, is the dense outer structure while the inner structure is cancellous bone. The diaphysis of long bones is mostly cortical bone, while there are significant amounts of cancellous bone in epiphyseal regions. The outer layer of cortical bone is the periosteum, a tough, fibrous, highly vascular membrane; tendons and ligaments attach to the outer layer of this membrane, The periosteum contains Volkmann's canals, small channels running

perpendicular to the diaphysis that incorporate blood and lymph vessels and nerves, directing them from the surface to the intracortical layer. This layer is arranged with structural units, the osteons, which have concentric layers, the lamellae, major contributors to the strength. Mature bone cells, the osteocytes, occupy spaces between the concentric layers of lamellae. These spaces are the lacunae, between which are canaliculi, in within which the osteocytes are networked to each other. A Haversian canal passes through the center of each osteon, which connect adjacent osteons and blood vessels to the periosteum. The inner layer of cortical bone is the endosteum, which consists of osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells) that are responsible for bone remodeling. The other type of bone is cancellous bone, often referred to as spongy or trabecular bone. Its structure is of a honeycomb nature, and it is found at the ends of most long bones and within many of the other bone types, including the ribs. The elements of bone within this structure are trabeculae, which are oriented along the lines of stress; if the biomechanical environment of a bone is altered, the trabeculae can slowly remodel to accommodate new stress patterns.

Bone is highly vascularized; blood vessels in bone are necessary for nearly all skeletal functions. Cortical bone is perfused by arterial blood originating from the main nutrient arteries as well as from smaller periosteal arteries, the blood flow in the long bones being largely centrifugal, radiating outward after delivery to the marrow cavity. The ends of long bones are supplied by the metaphyseal and epiphyseal arteries, which arise from the arteries from the associated joint. Each artery is accompanied by nerve fibers, which branch into the marrow cavities. Bone requires a substantial blood flow in order to supply the requisite oxygen and nutrients, and to eliminate metabolic waste products. The rich perfusion of bone reflects not only the significant requirements of bone cells, but also of the hematopoietic lineage cells, stromal cells and adipocytes of the bone marrow, and the endothelial cells. The vascular supply of bone enables rapid growth and remodeling, which is not possible in cartilage since, as discussed in the next section, it is essentially avascular. In consequence of this, should the blood supply to bone be disrupted, either through trauma, ageing or other processes, the bone can effectively die, a condition known as osteonecrosis, which is discussed later.

Critical to many of the characteristics and functions of bone are the mechanisms by which bone continually reconstructs itself and the role of bone cells in these processes. The rate of bone turnover is about 10% of the adult skeleton each year. This bone involves resorption followed by replacement with little change in shape. The resorption, which releases minerals and the transfer of calcium from bone to the systemic circulation, is the result of the activity of osteoclasts that attach to the osteons and secrete collagenase and other enzymes, which degrade the bone matrix. Osteoblasts are mature bone cells responsible for bone formation and ossification, producing the organic phase of the matrix, which subsequently is mineralized. This is a natural process, the characteristics of which are controlled by mechanical stress and metabolic factors. Without stress, (for example with prolonged bed rest or with astronauts in zero-gravity situations) bone resorption dominates. This also happens in some disease states, such as osteoporosis.

This bone remodeling phenomenon is vitally important in relation to the application of medical technologies to reconstruction of the musculoskeletal system. Conditions that affect human bones include those of arthritis, both osteo- and rheumatoid-, abnormal bone density and structure, including the osteoporosis mentioned above and its converse osteopetrosis, autoimmune conditions such as lupus, hyperthyroidism, genetic conditions such as osteogenesis imperfecta and Paget's disease and, of course, trauma. In many of these situations, remodeling is affected one way or another, or is crucial in healing processes. The two most relevant conditions are trauma, i.e., bone fracture, and arthritis.

Bone is one of the tissues that heals spontaneously and can do so without medical intervention. However, fracture healing is dependent on the activity of the bone cells already mentioned and in the geometrical characteristics of the fracture site. For optimal function results, it is necessary for the bone segments to be

properly aligned, which can occur naturally or with the assistance of external (splints, plaster casts) or internal (plates, rods, screws) support. Without this alignment, and without appropriate biomechanical characteristics, and without good osteoblast function, perfect results are rarely achieved. It should be noted that, in the vast majority of situations and patients, bone heals at a biologically determined rate, and no well-meaning interventions can accelerate this. In exceptional cases where healing is slow (delayed union) or impossible (non-union), some pharmacological or biophysical (e.g., electrical stimulation) may help, but even then, only with difficulty.

Arthritis involves the destruction of the bone and cartilage of certain joints, largely through ageing (osteoarthritis) or autoimmunity (rheumatoid arthritis). This is discussed at significant length elsewhere, the relevant point here being that the osteoclast – osteoblast interactions are crucial in allowing medical technologies to produce effective reconstructive solutions.

2.2.2.3.2 Cartilage

Cartilage is a versatile structure that occurs in many parts of the body, providing, for example, the flexible tissues of the nose and external ear. In the embryo, it is the precursor to bone and its flexibility and toughness are very advantageous in that capacity. Within the musculoskeletal system, it occurs in two forms, hyaline cartilage and fibrocartilage. The most common form is hyaline cartilage, a translucent, blueish-white, shiny connective tissue that is usually only 2 – 4 mm thick; almost unique among major connective tissue constituent, this cartilage is avascular, so that nutrients and oxygen must be obtained through diffusion. The collagen fibers are primarily type II. Fibrocartilage exists where tendons and ligaments are attached to bone, for example at the pubic symphysis and in the sternoclavicular joint, and it comprises the annulus fibrosus, which is the center of the intervertebral disc. It is a very strong and pliable connective tissue, being composed of both type II and type I collagen fibers. Aggrecan, a large chondroitin sulfate molecule is the predominant proteoglycan present in cartilage. Aggrecan self-assembles into a supramolecular structure with up to 50 monomers bound to a filament of hyaluronan. This provides the osmotic resistance necessary for cartilage to resist compressive loads.

There is significantly more extracellular matrix than cells in cartilage; the avascular nature and associated low oxygen environment do not allow for large cell numbers, and there is little metabolic activity. Because of this, there is little regenerative capacity in cartilage, which accounts for the destructive nature of cartilage arthritic conditions, and the difficulty in persuading regeneration within tissue engineering experiences. The ECM has several characteristic elements. The collagen matrix gives form and strength to cartilage tissue through a mesh-like structure of fibrils. There are also low levels of some non-collagenous elements of the ECM, such as decorin and versican.

Chondroblasts are the cartilage cells that produce the elements of the ECM. They form a matrix of hyaluronic acid, chondroitin sulphate, collagen fibers, and water, eventually becoming immobile aa they become surrounded by this matrix, then being referred to as chondrocytes. These are contained within spaces called lacunae. Within articular cartilage of the joints, chondrocytes increase joint articulation. They also regulate epiphyseal plate growth and assist chondroblasts in maintain the existing ECM. Fibroblasts are also present i**n** cartilage, producing type I collagen; in some situations, they are able to transform into chondrocytes.

There are several serious disorders and deficiencies of cartilage; indeed, cartilage diseases represent one of the most common groups of conditions that affect quality of life in developed countries[85]. Hyaline

[85] Krishnan Y and Grodzinsky AJ, Cartilage diseases, *Matrix Biology,* 2018;71-72:51-69. doi:10.1016/j.matbio.2018.05.005.

cartilage plays a crucial role in skeletal growth and development, first during endochondral bone formation in embryonic and fetal life and then in the form of growth plates which lead to the elongation of bones during childhood and adolescence. In addition to external injuries, genetic mutations can also affect growth plate cartilage disturbing normal skeletal growth; these genetic conditions are called chondrodysplasias. In a similar manner, osteochondritis dissecans is a focal, idiopathic alteration of subchondral bone with risk for instability and disruption of adjacent articular cartilage that may result in premature osteoarthritis. There are several inflammatory rheumatic diseases that lead to arthritis and can severely damage cartilage tissue. These include rheumatoid arthritis, juvenile idiopathic arthritis, gout, systemic lupus erythematosus, and seronegative spondyloarthropathies. As noted with respect to bone, osteoarthritis affects joint structure with involvement of both bone and cartilage. Tumors involving cartilaginous tissue constitute a major class of bone tumors. Benign cartilaginous tumors include osteochondroma, enchondroma, periosteal chondroma, multiple chondromatosis or enchondromatosis, chondroblastoma, and chondromyxoid fibroma. The epidemiology these tumors is uncertain since most cases remain undiagnosed due to the absence of symptoms. When they are symptomatic, benign tumors can present with pain, swelling, lesions or a pathological fracture. Malignant cartilaginous tumors include several types of chondrosarcomas.

Today, there is a marked absence of disease-modifying drugs related to cartilage conditions, and technologies that are aimed at cartilage reconstruction are very limited.

2.2.2.3.3 Joints

A joint is a structure that separates two or more adjacent elements of the skeletal system. Depending on the type of joint, these separated elements may or may not move on one another. Movement at joints may involve spinning, swinging, gliding, rolling, and approximation.

Spinning, commonly referred to anatomically as rotation, is the movement of a bone around its own long axis; for example, the radius of the forearm can rotate upon the lower end of the humerus in the upper arm at the elbow. Here, medial (inward) rotation of the radius gives pronation, while lateral (outward) rotation gives supination. Angular movement causes a change in the angle between the long axis of a moving bone and some reference line in a bone, as with flexion (bending) and extension (straightening) of the elbow. Any movement of one bone away from another is called abduction, while the reverse is adduction. Gliding and rolling actions cause a moving bone to swing.

Joints are either transient or permanent. The bones of the former fuse together at some point after birth, examples being many of the joints in the skull. Joints are also either described as diarthroses, in which fluid is present and which are freely movable or synarthroses, in which there is no fluid and the joints are immovable. Some classifications also include amphiarthroses, which are slightly movable, having cartilaginous tissue that holds the bones very tightly, as with the vertebrae.

Synarthroses are divided into fibrous and symphysis structures. In fibrous joints those parts that move are separated by collagen fibers, which pass from one part to the other. There are two types of fibrous joints, suture and gomphosis. Sutures are transient, being the unossified parts of the skeleton that become fused at various times after birth, being active sites of growth of the bones they separate. A gomphosis is a fibrous mobile peg-and-socket joint. The only examples are the roots of teeth, which fit into sockets in the mandible and maxilla, this being discussed in the next section.

Symphyses, or fibrocartilaginous joints, are where the body (physis) of one bone meets the body of another. All but two of the symphyses are found in the spinal column, and all but one contains fibrocartilage. The symphysis between the bodies of two adjacent vertebrae is called an intervertebral disc, which is discussed separately.

Diarthroses are commonly referred to as synovial joints, which consists of a wall enclosing a joint cavity that is wholly filled with synovial fluid. The wall has two layers, an outer complete fibrous layer, and an inner incomplete synovial layer. In a few diarthroses the fibrous layer also projects inward to become intra-articular disks, or menisci.

The fibrous layer is composed of collagen. Articular cartilage, which covers the articulating part is called hyaline because thin sections of it are translucent or transparent. The part of the cartilage nearest to the bone contains calcium phosphate crystals. There is a central articulating part and a marginal non-articulating part that is covered by a synovial membrane. The central part is either single, if only two bones are involved, or divided into distinct portions by sharp ridges, if more than two bones are included. For example, the upper articular surface of the humerus is single, for only this bone and the scapular are in the shoulder joint, but the lower articular surface of the humerus has two parts, one for articulation with the radius and one for articulation with the ulna, both being included in the elbow joint.

Chemically, synovial fluid is a dialysate of blood plasma, but it contains a larger amount of hyaluronic acid than other plasma dialysates. Physically, it is a markedly thixotropic fluid and viscoelastic. Functionally, it has roles in both nutrition and lubrication.

There are several types of synovial joint, the number and individual descriptions depending on the source of the information. The principal types of synovial joints are:

- The ball-and-socket joint, also known as a spheroidal joint, is the only one with three types of movement. The rounded surface of one bone moves within a depression on another bone, thus allowing greater freedom of movement than any other kind of joint. It is most highly developed in the large hip and shoulder joints of mammals, including humans.

- The hinge, or ginglymus, joint has each mating surface ovoid on its right and left sides, which allows backward-forward swing. The swing of the joint, however, differs from that of a conventional engineering hinge as it is accompanied by a slight rotation of the moving bone around its long axis. The joints between the phalanges of the fingers and that between the ulna and the humerus at the elbow are good examples.

- The plane, or arthrodial, joint has mating surfaces that are slightly curved and provide only a small amount of gliding movement. Examples are the joints between the metacarpal bones of the hand.

- The pivot, or trochoid, joints are of two forms: in one a pivot rotates within a ring; in the other a ring moves around a pivot. For example, the pivot joint between the atlas and the axis (first and second cervical vertebrae), directly under the skull, permits turning of the head from side to side.

- A condylar joint, sometimes called bicondylar, has two distinct surfaces on one bone that with corresponding distinct surfaces on another bone. These joints have two types of movement: one is always a swing, and the other is either another swing or a spin. The largest bicondylar joint is the tibiofemoral joint, in which both pairs of mating surfaces are within a single joint. Flexion and extension are the main movements but active rotation of the leg on the femur may be possible.

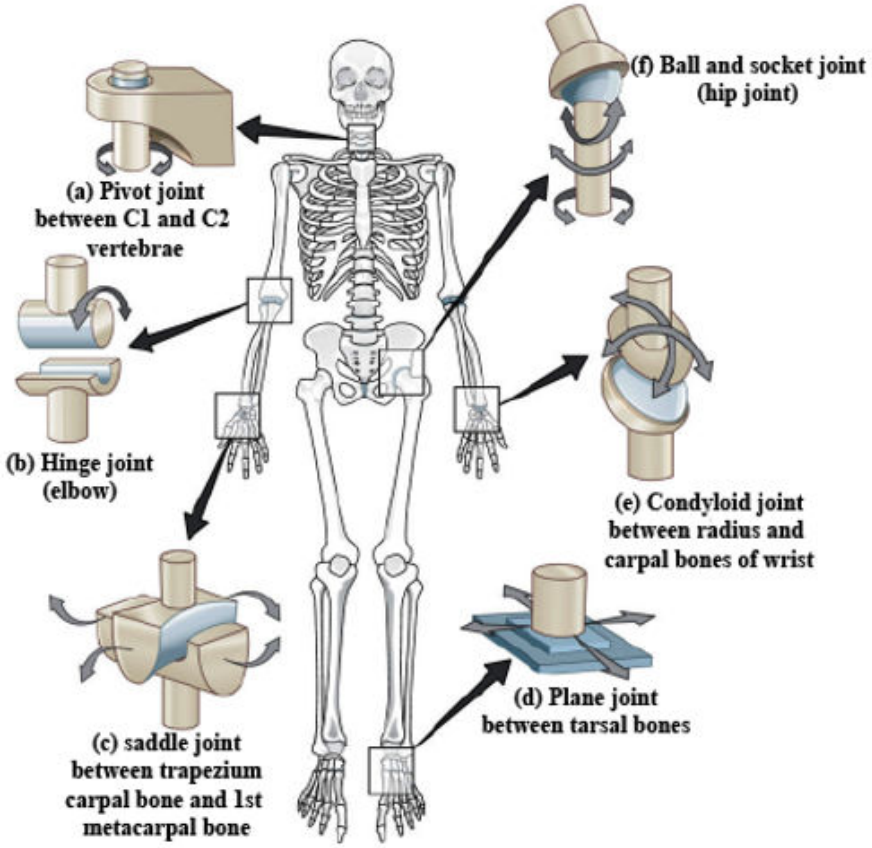

Figure 2.34. Examples of synovial joints in the human body.

Diseases, dysfunction, and abnormalities of joints arise from many sources, some very rare, some very common. They can be discussed briefly under the categories of genetic / developmental disorders, infectious diseases, cancer, arthritis-type conditions, and trauma. Genetic disorders of the skeleton involve a large group of rare but quite distinct conditions, the nature of which were presented in an excellent, if quite old, review by Kornak and Mundos[86] in 2003. The clinical diversity of these conditions makes them difficult to diagnose and classify; they are generally considered to be associated with either malformation of individual bones or of osseo-chondral tissues (i.e., cartilage – bone constructs), which manifest as abnormalities of broader structures such as joints. Much of the focus has been directed towards developmental hip dysplasia, previously called congenital dislocation of the hip. The genetic component is substantial[87]; however, there are many other factors including birth / delivery variables, birth weight, inflammation, and injuries. Treatments include braces or casts for the very young, osteotomy (surgical repositioning of the bones) for older people, and potentially total joint replacement. Other anatomical disorders that can have combined genetic and developmental aspects include femoro-acetabular impingement, where the bones of the joint are irregularly shaped and positioned such that painful

[86] Kornak U and Mundlos S, Genetic disorders of the skeleton: a developmental approach, *American Journal of Human Genetic*s, 2003;73:447-74.
[87] Harsanyi S, Zamborsky R, Kokavec M *et al*, Genetics of developmental dysplasia of the hip, *European Journal of Medical Genetics*, 2020;63:103990. doi:10.1016/j.ejmg.2020.103990.

articulation occurs, treatable by carefully controlled physical exercise, various anti-inflammatory agents and arthroscopic correction of surfaces and structures.

In children, Legg-Calve-Perthes disease is caused by inadequate blood supply to the femoral head. Avascular necrosis is somewhat similar, occurring in more elderly individuals, with death of the bone, typically within the femoral head and neck, arising from a number of causes.

Arthritis is the most common type of disease that affects joints, especially the synovial joints; it is prevalent world-wide and places a very high demand of healthcare systems. There are two forms, rheumatoid and osteoarthritis. Rheumatoid arthritis is an autoimmune disorder which induces chronic inflammation in joints and also in a wide variety of other tissues and systems, including the skin, lungs, heart and blood vessels. Rheumatoid arthritis affects the lining of joints, causing a painful swelling, bone erosion and joint deformity. The broader aspects of autoimmunity are discussed later in the book.

The inflammation associated with rheumatoid arthritis can damage other parts of the body as well. While new types of medications have improved treatment options dramatically, severe rheumatoid arthritis can still cause physical disabilities. Early stages of the disease affect smaller joints, particularly in the hands and feet. Symptoms may then spread (usually bilaterally) to the wrists, knees, ankles, elbows, hips and shoulders. These symptoms may vary in severity over time, with periods of increased and disease activity. Women are more likely than men to develop rheumatoid arthritis and it most commonly begins in middle age. Individuals with rheumatoid arthritis have an increased risk of inflammation and scarring of the lung tissues, and a higher risk of lymphoma.

Remission of symptoms should occur with the early use of so-called disease-modifying antirheumatic drugs (DMARDs). Common DMARDs include methotrexate, leflunomide and sulfasalazine (Azulfidine). A more recent type of drug is the biologic response modifier, including abatacept, golimumab and infliximab, which are often prescribed alongside methotrexate. This type of drug also increases the risk of infections. In addition, nonsteroidal anti-inflammatory drugs (NSAIDs) can relieve pain and reduce inflammation, while corticosteroid medications, such as prednisone, reduce inflammation and pain and slow joint damage. Surgery may be used if medication is ineffective, including synovectomy**,** which the inflamed lining of the joint, joint fusion that stabilizes or realigns a joint, or total joint replacement.

Osteoarthritis involves the gradual deterioration of the cartilage within joints, producing pain, joint stiffness, loss of flexibility and soft tissue inflammation and swelling. Osteoarthritic changes eventually affect the entire joint and deterioration of the connective tissues that hold the joint together. Risk factors include older age, obesity, trauma, repetitive stresses on the joint, bone deformities, some metabolic diseases, and genetic factors.

Relief of osteoarthritis symptoms include the use of pain modifiers such as acetaminophen and non-steroidal anti-inflammatory agents. Physical and occupational therapies may help, but sooner or later, invasive options may become necessary. These include injections of a corticosteroid or a hyaluronic acid lubricant. Surgically, total joint replacements are very popular (discussed in detail elsewhere), but it is also possible to realign bones by techniques of osteotomy.

2.2.2.3.4 Spirituality and cultural aspects on the human bones and joints

(To be completed, Q1, 2024)

2.2.2.4 Teeth and Parodontal Tissues

Teeth are contained within the oral cavity, commonly referred to as the mouth. This is mostly filled with the tongue (see earlier) and is bounded anteriorly and on the sides by the alveolar process containing the teeth and posteriorly by the isthmus of the fauces, which opens into the throat. The roof of the mouth is the hard palate, and the posterior is the soft palate. The inner lining of the mouth, the oral mucosa, is composed of stratified squamous epithelium, and several salivary glands secrete viscous and mucoid fluid into the mouth for lubrication and moisture control.

In this section, I specifically deal with the teeth and the tissues that surround them; such tissues may be referred to as parodontal tissues or the periodontium. A tooth consists of a crown, the functional part that is visible above the gum, and one or more roots, which are normally unseen, being attached to the alveolar processes of the jaws by a fibrous periodontal ligament. In humans, and other mammals, the tooth consists of three layers, an outer layer of enamel in the crown and of cementum in the root, a middle layer of dentin and the innermost part, the pulp.

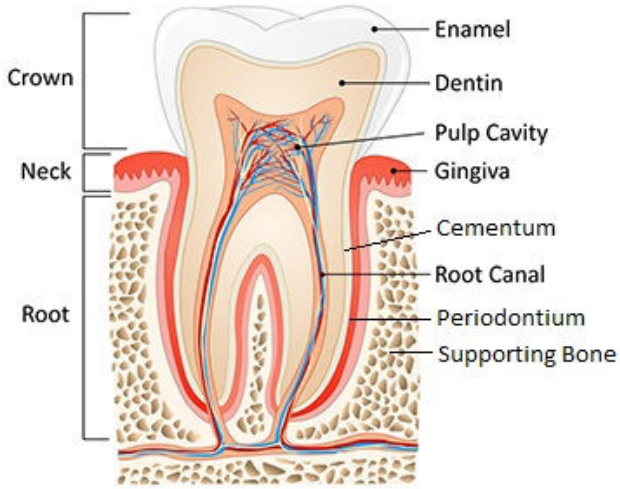

Figure 2.35. Structure of human tooth and periodontium.

The enamel is the hardest and most mineralized tissue in the human body[88]. It consists of over 95% carbonated apatite, a form of calcium phosphate, whose elongated crystals align parallel to one another and are held together by a self-assembled organic glue. The dentin forms the bulk of the tooth structure[89]. Overall, it is less mineralized than enamel but more so than bone or cementum; it is, however, a complex tissue, with considerable variation in morphology. It is essentially a hydrated composite, comprising of oriented tubules surrounded by a highly mineralized peritubular zone that is embedded in a matrix of collagen (with some non-collagenous proteins) and apatite crystals, all zones containing dentinal fluid. The tubules have irregular walls with many interconnecting lateral branches. They contain the fluid as well as odontoblast processes; the fluid is able to move freely, and is associated with pain and sensation. The dental pulp contains connective tissue, mesenchymal cells, neural fibers, blood vessels and lymphatics; its main functions are to produce dentin and maintain the vitality of the dentin. Blood vessels

[88] Welborn VV, Enamel synthesis explained. *Proceedings of the National Academy of Science*s, 2020;117(36):21847-8. doi:10.1073/pnas.2014394117.
[89] Goldberg M, Kulkarni AB, Young M, *et al*, Dentin: Structure, composition and mineralization, *Frontiers in Bioscience (Elite Edition)*, 2012;3:711-35.

and nerve bundles enter the dental pulp through the apical foramen and provide nutrition and a responsive sensory nervous system. Unlike bone, which can remodel and repair, teeth do not readily undergo complete regeneration and demonstrate limited reparative processes. After the crown is formed, ameloblasts undergo programmed cell death and lose the ability to repair enamel *in vivo.* Although odontoblasts cannot repair dentin, their progenitor or stem cells are capable of migrating into the dentin surface and form reparative dentin. Unlike primary dentin, this reparative dentin is poorly organized, with irregular dentinal tubules embedded in the dentin matrix, but it provides a protective barrier to the dental pulp. The ability to create new odontoblasts throughout life in response to damage is associated with stem cells within the dental pulp.

Humans have two successive sets of teeth during life, with first a set of 20 primary and then 32 permanent teeth. The primary dentition, consisting of four incisors, two canines and four molars in each jaw, is complete by 2 to 3 years of age, shedding usually being finished by 13. The primary molars are replaced in the adult dentition by bicuspid premolars, giving a total of 32 teeth in the permanent dentition. Incisors are adapted for cutting, tearing, and holding objects, especially food; the upper incisors have a delicate tactile sense that enables them to identify such objects. Next to the incisors on each side is a canine tooth, also with the function of cutting and tearing food. Premolars and molars have a series of cusps used for breaking up particles of food. The number of roots for each type of tooth varies from one for incisors, canines, and premolars to two or three for molars.

The periodontal ligament is the specialized connective tissue that is placed between the cementum lining of the tooth root and the surrounding alveolar bone. It protects, supports, and provides sensory input to the masticatory system and maintains homeostasis in that region. It contains fibers, cells, blood vessels and nerves. The principal fibers are bundles of collagen, the orientation of which varies according to location and mechanical function. Most fibers have oblique orientations in order to resist the vertical forces of mastication. These fibers have extensions at both ends, known as Sharpey's fibers, which insert themselves into either the cementum or alveolar bone. Fibers are embedded in a ground substance, typical of the ECM of most soft tissues, consisting largely of non-collagenous proteins. The main cells of the ligament are fibroblasts, cementoblasts, osteoblasts and progenitor / stem cells. The ligament is well vascularized, the principal blood supply being derived from superior and inferior alveolar arteries. Innervation arises from the trigeminal nerve.

Teeth may be subject to irregularities in their alignment, especially in the relationship between the teeth in opposing jaws. This may lead to significant functional deficits, but usually misalignment is a matter of aesthetics; the clinical area of orthodontics is concerned with the correction of alignment errors for either functional or aesthetic reasons, or both.

Tooth decay, or dental caries, is common, but less so than a few decades ago because of far better oral hygiene and preventive measures. It originates from plaque, a film which builds up on the enamel surface that supports the activity of bacteria, which ferment sugar and starchy-food debris The result is the formation of acids that demineralize and destroy the enamel and then the underlying dentin. Caries is treated by removing affected tissue and replacement with filling materials. Such techniques are referred to as restorative dentistry and the substances used are among the most common biomaterials for reconstructing the body. The plaque that forms on tooth surfaces can also extend to the surrounding soft tissue, primarily the gingivae, causing inflammation and bleeding. This gingivitis, or periodontal disease, is a major cause of tooth loss.

2.2.2.4.1 Aesthetics and spirituality related to teeth

Confucius apparently said that '*behind every smile there's teeth*'. This is undoubtedly true, and the prominent position of the lips and teeth in the visible face has been the focus of attention for those concerned with the role of aesthetics in medical and dental care for millennia. The ambiguity of the smile

of Leonardo da Vinci's *Mona Lisa*, which famously does not show any teeth, has been the subject of speculation ever since it reached the public's eye.

Extending the discussion of aesthetics for a moment, it is interesting to follow the course of reconstructing teeth over those millennia, with both the clinical and spiritual aspects. Many essays have been written about ancient dental practices[90] and, as most authors will readily admit, interpretation of the evidence is not always straightforward. In the Neolithic city of Mehrgarb, located in modern-day Pakistan, evidence from excavated skeletal remains showed that quite sophisticated dental procedures, with flint-based drills, were used between 7,500 and 9,000 years ago[91]. It seems clear that since these procedures were often noted on parts of teeth deep within the jaw, mainly in molars, which were not easily visible, these treatments could not have been aesthetically motivated. In a study of dentistry in ancient Egypt, Forshaw refers to Hesyre as '*the earliest recorded dentist in the world'*, living around 2660 BC, in the area of Saqqara, near modern-day Cairo[92]. Evidence from skeletal and mummified remains shows quite an extensive array of dental artefacts and procedures. However, what we might refer to as forensic investigations have concluded that these did not necessarily relate to living subjects, for example because of the then unavailability of complex tools to carry out drilling with complex geometries, but rather were performed later, during the mummification process, to make the body more whole for the afterlife. Again, here, the evidence is rather vague since, as noted by Seiler and Ruhli[93], the process of mummification often included rough physical techniques to open mouths in order to release internal organs, which frequently resulted in extensive facial fractures; the beautification of some teeth hardly seems relevant and, in any case, the appearance of the mummy was far more represented by the use of elaborate shrouds.

What appears to be a bizarre concept, that of the tooth worm, has been a matter of speculation since Roman times and the Middle Ages[94]. It was believed by many people that parasites, labelled as worms, caused periodontitis and caries, and early dental therapies were aimed at the destruction of such worms. Equally, however, many did not believe in the physical presence of such worms, but did believe that they represented demons, and a source of evil; many superstitions surrounded this belief (in many parts of the world) and part of some dental practices were aimed at eradication of these demons.

Ohaguro is the ancient Japanese custom of blackening teeth. Excavated bones and clay figures from the Kofun period, called haniwa, were found with traces of blackened teeth, and this art held a prominent place in Japan's history. One of the main reasons for ohaguro is that pitch black objects were regarded as very pretty. Using the solution kanemizu, ferric acetate from iron filings mixed with vinegar and tannin, the custom was first used to celebrate a coming of age, to show that they had become adults. Importantly, however, the ohaguro process enhanced the acid resistance of the teeth and tightened the gingiva, minimizing tooth decay and periodontal disease. It was mostly used in young women and there is good evidence that their dental health was better than that of comparable males[95]. Ohaguro was banned by the Meiji government in 1870.

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[90] Diaconu D, Vitalariu A, Cotaie *et al,* Religious and spiritual concepts in dental practices in ancient orient, *International Journal of Medical Dentistry,* 2014;4(4):255-9.

[91] Coppa A, Bondioli L, Cucina A, *et al,* early Neolithic tradition of dentistry, *Nature,* 2006;440:755-6.

[92] Forshaw RJ, The practice of dentistry in ancient Egypt, *British Dental Journal,* 2009;206:479-84. doi:10.1038/sj.bdj.2009.355.

[93] Seiler R and Ruhli F, "The Opening of the Mouth" – a new perspective for an ancient Egyptian mummification procedure, *The Anatomical Record*, 2015;298:1208-16. Doi:10.1002/ar23140.

[94] Gerabek WE, The tooth worm: historical aspects of a popular medical belief, *Clinical Oral Investigations*, 1999;3:1-6.

[95] Oyamada J, Kitagawa Y, Hara M *et al,* Sex difference of dental pathology in early samurai and commoners at Kokura in Japan, *Odontology*, 2017;105:267-74.doi:10.1007/s10266-016-0275-0.

2.2.2.5 The Vascular System

Outside of the heart, the circulatory system of the human body has two major parts, the systemic and pulmonary vascular systems. The blood vessels that carry blood away from the heart (in both systems) are the arteries, while those that return blood to it are the veins. The arteries and veins interface within the capillary networks throughout the body and lungs.

2.2.2.5.1 Arteries

The largest and most important artery is the aorta. It comprises the ascending aorta which distributes oxygen and nutrients to the heart via the coronary arteries, the aortic arch, which has three major branches (the brachiocephalic trunk, the left common carotid artery, and the left subclavian artery) to direct blood to the upper body, and the descending aorta which channels blood to the torso, abdomen, and lower body. The aorta is referred to as the thoracic aorta above the diaphragm, below which it is the abdominal aorta.

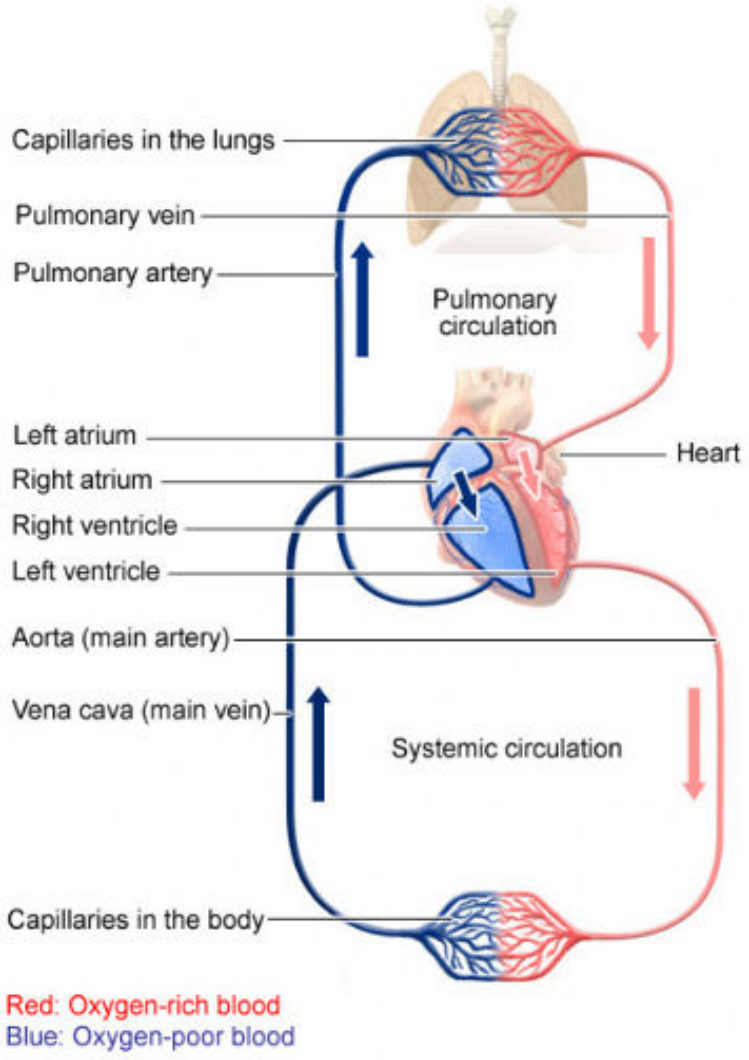

Figure 2.36. The circulatory system of the human body.

In the head and neck are the left and right common carotid arteries, the former coming directly off the aortic arch and the latter from the brachiocephalic trunk. There are two paired external carotid arteries, derived from the common carotid arteries, that supply blood to the face, lower jaw, and neck. Paired internal carotid arteries are the primary sources of blood for the brain. Paired vertebral arteries, derived from the subclavian arteries, travel up the neck, supplying additional blood to the brain., while the thyrocervical trunk branches into several vessels that direct blood to the thyroid, neck, and upper back.

In the torso, or trunk, are the two bronchial arteries that supply blood to the lungs, the esophageal artery supplying the esophagus, the pericardial artery that supplies the pericardium, and the intercostal and superior phrenic arteries that provide blood to the vertebrae, spinal cord, back muscles, diaphragm, and skin. The abdominal arteries include the celiac trunk which divides into smaller arteries that supply the stomach, liver and spleen, the superior and inferior mesenteric arteries that supply the pancreas, intestines and rectum, the renal arteries that deliver blood to the kidneys and the gonadal arteries, which are paired arteries that send blood to the testes or ovaries. The common iliac artery is a branch of the abdominal aorta that divides into the internal and external iliac arteries. The former supplies the bladder, pelvis, and external portion of the genitals, while the latter eventually becomes the femoral artery.

The arteries of the arm are the axillary artery, which is the name given to the subclavian artery as it exits the torso, the brachial artery that delivers blood to the upper region of the arm, and the radial and ulnar arteries that run alongside the two bones of the forearm where they eventually divide to deliver blood to the wrist and hand. Leg arteries include the femoral artery that supplies blood to the thigh and divides into the various smaller arteries. It becomes the popliteal artery below the knee, which divides into anterior and posterior tibial arteries.

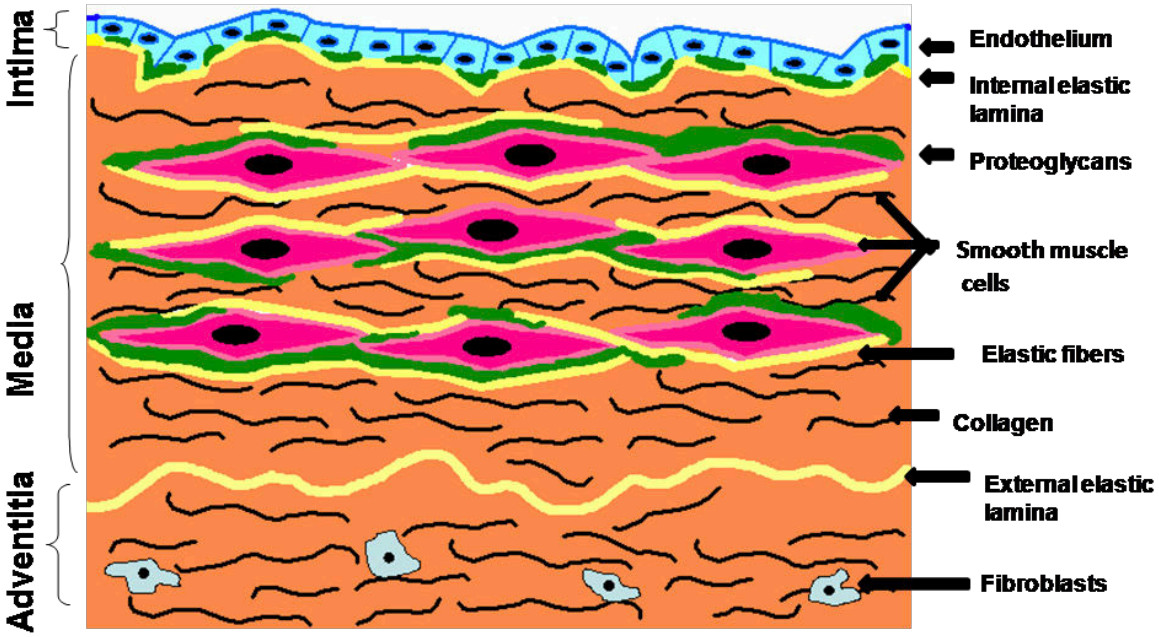

Figure 2.37. Structure of human arterial wall.

The maximum systolic blood pressure in humans should be 100-140 mm Hg and the minimum diastolic pressure between 60-90 Hg. On the other hand, the maximum pressure in veins should be 5-15 mm Hg. This implies that the mechanical properties of the blood vessel walls should be quite different, arteries needing to be more robust; it also follows that the structure and mechanical properties of arteries will vary with anatomical location, those nearer the heart experiencing higher pressures.

The arterial wall has three distinct layers, with an intima on the inner, or luminal surface, the media as the middle layer and an outer layer of the adventitia[96]. The intima is a single layer of endothelial cells bound to a basement membrane, which is separated from the media by elastic lamella. The media is composed of elastic fibers and smooth muscle cells. Collagen fibers dominate the adventitial layer, along with some fibroblasts. The hemodynamic factors related to artery location and size control the relative abundance and orientation of the collagen and elastic fibers.

The mechanical characteristics of arteries show distinct contributions from elastic and collagen components.

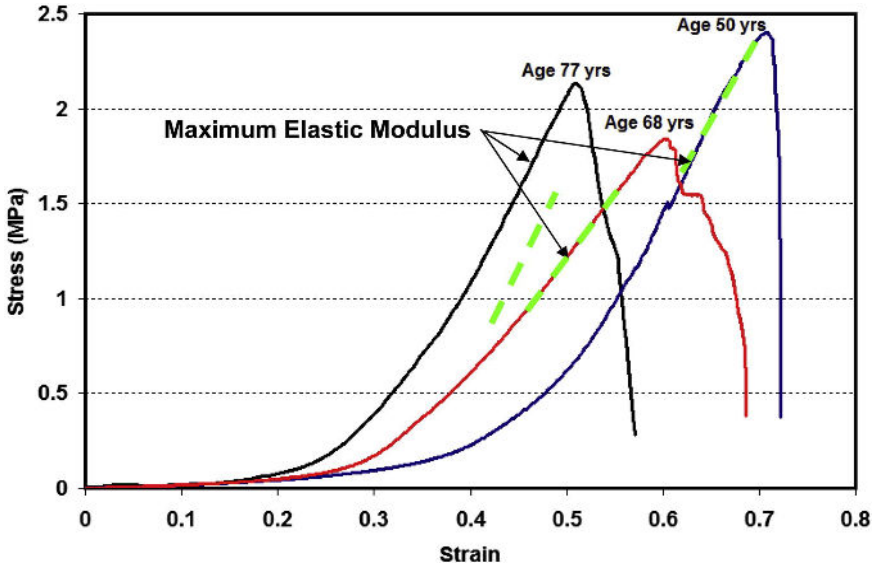

Figure 2.38. Stress – strain curve for human aorta, showing increasing stiffness with age[97].

At low values of stress, the elastic fibers contribute most to the deformation, but then collagen dominates the deformation characteristics, which alter with age. With the blood pressures mentioned above, under normal physiological conditions, vessels such as the aorta operate in the elastic region, with a modulus of elasticity in the region of 0.2 to 0.6 MPa and, in young healthy individuals, the behavior is essentially linear up to 40% strain. Above this level, the behavior is viscoelastic, that is with characteristics of both elastic solids and viscous liquids, in which the deformation is controlled by interactions between collagen fibrils and the behavior of smooth muscle cells.

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[96] Ebrahimi AP, Mechanical properties of normal and diseased cardiovascular system, *Journal of Vascular and Interventional Radiology*, 2009;2(2):155-62.

[97] Khanafer K, Schlicht MS and Berguer R, How should we measure and report elasticity in aortic tissue? *European Journal of Vascular and Cardiovascular Surgery*, 2013;45(4):332-9. doi.org/10.1016/j.ejvs.2012.12.015

2.2.2.5.2 Veins

There are four main types of vein. Pulmonary veins carry oxygenated blood from the lungs to the left atrium of the heart. Systemic veins return oxygen-depleted blood from the rest of the body to the right atrium. Superficial veins are found close to the surface of the skin without any relationship to a particular artery, while deep veins are located deep within muscular tissue, usually near a corresponding artery.

There are two internal and two external jugular veins, the former being responsible for receiving the majority of the blood coming from the brain. It has a larger diameter compared to the narrower external jugular, which is more superficial, draining blood from the scalp and parts of the face. There are two brachiocephalic veins, which form a V shape, receiving blood coming from the jugular veins and the subclavian veins. Several small veins in the lower leg terminate in the popliteal vein, behind the knee, which drains into the large femoral vein in the upper leg, which becomes the external iliac vein. Many of the veins in the legs are relatively small, in contrast to the great saphenous vein, which runs from the foot up to the hip joint, being the longest vein in the body, and, as we shall see, a very useful source of blood vessel grafts, The inferior vena cava is a large retroperitoneal vessel formed by the confluence of the right and left common iliac veins; it is responsible for transporting deoxygenated blood from the lower extremities and abdomen back to the right atrium of the heart.

Interspersed throughout the veins are valves, small crescent-shaped flaps of tissue that help prevent a backflow of blood. They project from the innermost layer of the vein wall to the center of the vein and are angled towards the heart in the direction of venous blood flow. As blood flows past a valve, it pushes the valve open and as the blood flow slows, the valve swings back to its closed position.

2.2.2.5.3 Capillaries

Capillaries are very small blood vessels that connect arteries and veins and facilitate the exchange of certain components between blood and tissues. Tissues that are very metabolically active, such as muscles, liver and kidneys, have an abundance of capillaries. The exchange of oxygen, nutrients, and waste between blood and tissues occurs within capillaries, either by passive diffusion, the movement of a substance areas of high to lower concentration, or by pinocytosis, where cells actively take in small molecules, such as fats and proteins. The walls of capillaries are made up of a thin endothelial layer which is surrounded by a basement membrane. Their single-layer structure makes capillaries somewhat leaky, allowing easier passage of oxygen and other molecules, and allowing passage of immune cells to reach sites of infection or inflammatory damage.

There are three types of capillaries. Continuous capillaries are the most common; they contain small gaps in between their endothelial cells that allow relatively easy passage of some substances The continuous capillaries in the brain do not have such gaps, enhancing the protective function of the blood-brain barrier. Fenestrated capillaries are leakier as they contain small pores; they are especially found in the small intestine and the kidneys. Sinusoid capillaries are the rarest and “leakiest” type, which allow for the exchange of large molecules or even cells through many larger gaps in their capillary wall; the surrounding basement membrane is also incomplete with openings in many places. These are found in tissues, such as the liver, spleen, and bone marrow.

2.2.2.5.4 Diseases and dysfunction in the vascular system

Not surprisingly, many parts of the vascular system are susceptible to disease, some conditions being rare and quite easily managed and others leading to failure or collapse of the circulation, often with fatal consequences. The arterial system is most vulnerable, the main conditions arising either from weakening of the vessel wall or progressive narrowing of the lumen caused by deposition of atherosclerotic plaque.

A weakening of the arterial wall is referred to as an aneurysm, seen most profoundly in the aorta. An aortic aneurysm is a bulge in the wall of the aorta and can lead to aortic rupture and aortic dissection. A rupture is a complete tear through all three layers of the aorta; blood bursts through the hole into the surrounding tissues. An aortic dissection begins abruptly when a tear occurs in the inner layer of a weakened area of the aorta, blood surging through the tear, causing the inner and middle layers to separate. The normal blood flow in the vessel lumen may be slowed or stopped, or the aorta may rupture completely. This is a life-threatening condition. The pathophysiology of the disease is related to an initial arterial insult causing a cascade of inflammation and extracellular matrix protein breakdown by proteinases. Risk factors include cigarette smoking, advanced age, dyslipidemia, hypertension, and coronary artery disease.

Abdominal aortic aneurysms ("AAA" or "Triple A") occur the segment of the aorta within the abdominal cavity. Thoracic aortic aneurysms are found within the chest and may be classified as ascending, aortic arch, or descending aneurysms. Thoracoabdominal aortic aneurysms involve both the thoracic and abdominal aorta. AAAs are more common than their thoracic counterpart since elastin is reduced in the abdominal aorta as compared to the thoracic aorta. The prevalence of AAAs increases with age, with an average age of 65–70 at the time of diagnosis. Before rupture, an AAA may present as a large, pulsatile mass above the umbilicus. Unfortunately, rupture may be the first hint of AAA. Once an aneurysm has ruptured, it presents with classic symptoms of abdominal pain which is severe, constant, and radiating to the back. The risk of rupture of an AAA is related to its diameter; once the aneurysm reaches about 5 cm, the risk may exceed the risks of surgical repair. Less than 25% of patients survive rupture due to large pre-and postoperative mortality. Annual mortality from ruptured aneurysms in the USA is about 15,000. The management of patients with aortic aneurysms, reserved for smaller aneurysms or frail patients, involves cessation of smoking, blood pressure control, use of statins and drugs such as beta blockers. Decisions about repairing an aneurysm are based on the balance between the risk of rupture without treatment and the risks of the treatment itself. Details of repair processes are given in a later Chapter.

As with the blood vessels of the heart (i.e., the coronary arteries), peripheral arteries (i.e., outside the heart) also may develop atherosclerosis, associated with fatty deposits, called plaque, on the inside walls. The narrowed artery causes less blood to flow, creating the condition of ischemia. When this peripheral artery disease occurs within the legs, it can lead to leg pain or cramps, known as claudication, along with changes in skin color, sores, or ulcers. Total loss of circulation can lead to gangrene, necessitating amputation. A blockage in the renal arteries may cause renal artery disease, with uncontrolled hypertension, abnormal kidney function and congestive heart failure. Carotid artery disease is a narrowing in the arteries supplying the brain, possibly leading to a transient ischemic attack or stroke. Aneurysms and dissections can also occur in the carotid arteries.

Venous disease is generally less common and less problematic than arterial disease. Varicose veins are enlarged, twisted veins, which can happen anywhere in the body, but most commonly in the legs. They are not considered a serious medical condition but can be uncomfortable and lead to more serious problems. Chronic venous insufficiency is a condition that occurs when the venous wall and/or valves are not working effectively, making it difficult for blood to return to the heart.

2.2.2.6 Lymphatic System

I will deal with the lymphatic system only briefly since it is not very relevant to current concepts of reconstruction. Although the presence of a circulatory system separate from the main cardiovascular system had been suspected for some time, it was not until the 19th century that the anatomy of the

lymphatic structures was established[98]. The system is only found in vertebrates, where it exists in all compartments except neural tissue and bone marrow. It is composed of draining lymphatic vessels, lymph nodes and associated lymphoid organs (e.g., tonsils, spleen, and thymus). The vessels are blind-ended unidirectional absorptive channels that transport interstitial fluid, immune cells and macromolecules to the lymph nodes and, from there, to the venous circulation *via* the thoracic duct and the right lymphatic trunk.

The main physiological function of the lymphatic vasculature is to take up fluid, leaking out of blood capillaries into interstitial spaces in the tissue, and to return it to the blood circulation; it also protects against foreign antigens. Failure to do this effectively results in lymphedema, a chronic, disabling and disfiguring condition[99]. The accumulation of protein-rich fluid in the tissues causes swelling of the extremities and is in most patients also associated with inflammatory reactions, fibrosis, overgrowth of adipose and connective tissue in the affected areas. Based on its cause, lymphedema can be classified into primary (hereditary) and secondary (acquired) forms. Primary lymphedema is rare and generally characterized by hyper- or hypoplastic lymphatic vessels. Secondary lymphedema develops upon obstruction of lymphatic vessels, which is mostly the result of surgery or radiotherapy for breast cancer. In tropical countries lymphatic filariasis, a mosquito-borne parasitic infection, is the most common cause of lymphedema.

No curative treatment for lymphedema exists, and therapeutic measures are limited to manual lymph drainage and compression bandaging. The lymph nodes provide a focal point for intervention in the lymphatic system. Lymph node dissection, or lymphadenectomy, is a surgical procedure in which the lymph nodes are dissected, and a sample of tissue is checked for the presence of malignancy. The four most common dissection sites are axillary lymph nodes (for breast cancer), inguinal lymph nodes (for penile, anal, and vulvar cancers), cervical lymph nodes (for head/neck cancers and thyroid cancers), and retroperitoneal lymph nodes (for testicular and ovarian cancers). Secondary lymphedema is a common occurrence after such dissections[100]. There are some treatments, such as lymph node bypass, that can be used in patients who have the condition, but it appears possible, using microsurgical or even robotic techniques, to prevent lymphedema when used at the time of the original dissection.

2.2.2.7 Gastrointestinal System

The GI tract is a series of hollow organs that are connected to each other, extending from the mouth to the anus, via the esophagus, stomach, small intestine and large intestine. Some major organs are associated with this tract, such as the liver, which collectively constitute the digestive system. Such organs have been discussed previously, as have parts of the mouth. The discussion here starts with the pharynx and esophagus.

2.2.2.7.1 Esophagus

The esophagus is a dynamic, flattened muscular tube, 18 to 26 cm long in the adult, that pushes food toward the stomach, where digestion and absorption take place. Mucus produced by the esophageal mucosa provides lubrication and active peristaltic contractions propel residual material from the esophagus into the stomach. Between swallows the esophagus is collapsed but the lumen can distend to approximately 2 cm in the anterior-posterior dimension and up to 3 cm laterally to accommodate a

[98] Aspelund A, Robciuc MR, Karaman S, *et al,* Lymphatic system in cardiovascular medicine, *Circulation Research,* 2016;118:515-30, doi; 10.1161/CIRCRESAHA.115.306544.
[99] Cueni LN and Detmar M, The lymphatic system in health and disease, *Lymphatic Research and Biology*, 2008;6:109-22, doi:10.1089/lrb.2008.1008.
[100] Coriddi M, Mehrara B, Skoracki R, *et al,* Immediate lymphatic reconstruction: Technical points and literature review, *Plastic and Reconstructive Surgery Global Open*, 2021;9:e3431, doi:10.1097/GOX.0000000000003431.

swallowed bolus. Beginning in the neck, at the pharyngoesophageal junction, the esophagus descends anteriorly to the vertebral column and after traversing the diaphragm, it extends through the gastroesophageal junction to end at the orifice of the cardia of the stomach. There are three distinct regions: cervical, thoracic, and abdominal. Structurally, the esophageal wall is composed of four layers: innermost mucosa, submucosa, muscularis propria, and adventitia. Two high-pressure zones prevent the backflow of food: the upper and lower esophageal sphincter. These functional zones are located at the upper and lower ends of the esophagus.

Branches of the inferior thyroid artery provide arterial blood supply to the upper esophageal sphincter and cervical esophagus. Paired aortic esophageal arteries supply the thoracic esophagus. The arteries supplying the esophagus end in an extensive, dense network in the submucosa. From the dense submucosal plexus, the venous blood drains into the superior vena cava. The veins of the proximal and distal esophagus drain into the azygous system. Collaterals of the left gastric vein receive venous drainage from the mid-esophagus. The submucosal connections between the portal and systemic venous systems in the distal esophagus form esophageal varices in portal hypertension. These submucosal varices are sources of major hemorrhage in conditions such as cirrhosis.

Tracheoesophageal fistula and esophageal atresia are the most frequent congenital esophageal abnormalities, the former resulting from defects in the separation of the respiratory tract from the foregut and the latter arising from failure of the primitive gut to recanalize during embryonic development. Congenital esophageal stenosis is a narrowing of the esophageal lumen, most frequently seen in the distal third. A variety of disorders may affect the esophagus, the most common being gastroesophageal reflux disease, GERD, which occurs when the lower esophageal sphincter does not close properly and stomach acid and contents flow backwards. In contrast, achalasia occurs when that sphincter does not open or relax, preventing food entering the stomach. Esophageal structures arise when the esophagus becomes too narrow, allowing only slow transport of food. Hiatal hernias occur when the upper part of the stomach protrudes above an opening in the diaphragm, leading to greater levels of reflux. For those with chronic, untreated acid reflux, the lining of the lower part of their esophagus resembles the stomach lining. This condition, known as Barrett's esophagus, is associated with a higher risk of esophageal cancer. There are two types of esophageal cancer, squamous cell carcinoma and adenocarcinoma. People who ingest caustic substances, i.e., those with pH less than 2 or greater than 12) experience severe injury in the esophagus. Treatment options for esophageal diseases have improved in recent years[101]. This has occurred through the use of minimally invasive and robotic techniques for tissue resection, since classical therapeutic options, for example with proton pump inhibitors have proved rather ineffective. Many techniques are variations of esophagectomy, which is the surgical removal of part of the esophagus and reconstruction using a graft from elsewhere in the GI tract.[102] These may be used for cancer treatment as well as some of the other conditions, but mortality rates are quite high.

2.2.2.7.2 Stomach

There are four main regions in the stomach. At the cardia**,** the esophagus connects to the stomach. Above and to the left of the cardia, is the dome-shaped fundus**,** below which is the main part of the stomach. A funnel-shaped pylorus connects the stomach to the duodenum. The smooth muscle pyloric sphincter is located at this point, and it controls stomach emptying. In the absence of food, the stomach deflates inward, and falls into large folds called rugae. The stomach is held in place by the lesser omentum, which extends from the liver and the greater omentum, which runs to the posterior abdominal wall.

[101] Martinek J, Aklyama J-I, Vackova Z, *et al,* Current treatment options for esophageal diseases, *Annals of the New York Academy of Sciences,* 2016;1381(1):139-51. doi:10.1111/nyas.13146.
[102] Flanagan JC, Batz R, Saboo SS, *et al,* Esophagectomy and gastric pull-through procedures: Surgical techniques, imaging features and potential complications, *RadioGraphics*, 2015;36:107-21, doi:10.1148/rg.201650126.

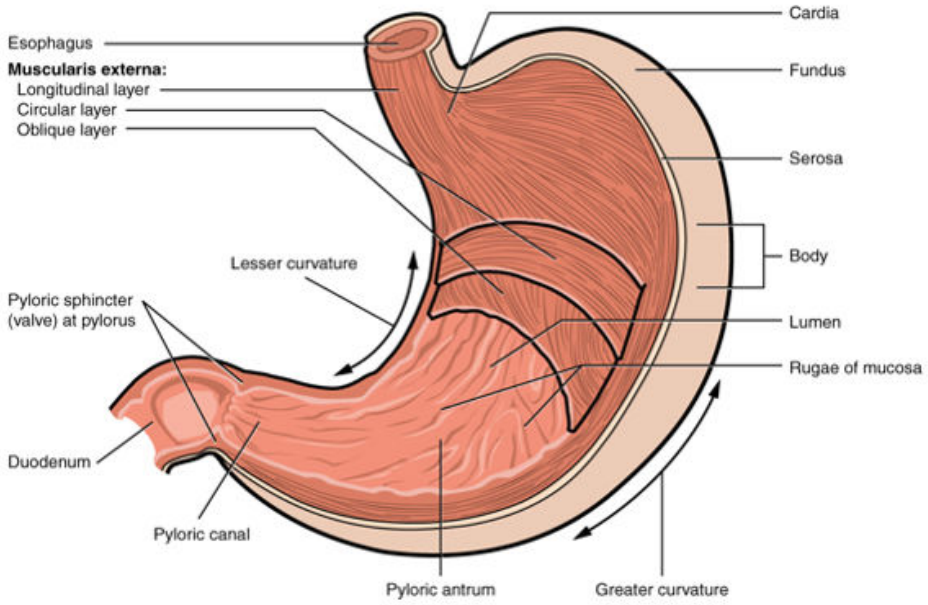

Figure 2.39. Structure of the human stomach.

The wall of the stomach is similar to that of the rest of the GI tract, but with adaptations to the mucosa and muscularis for its unique functions. In addition to the typical circular and longitudinal smooth muscle layers, the muscularis has an inner oblique smooth muscle layer so that in addition to moving food forward, it can mechanically break it down into smaller particles. The stomach mucosa's epithelial lining consists only of surface mucus cells, which secrete a protective coat of alkaline mucus. A large number of gastric pits cover the surface of the epithelium, each marking the entry to a gastric gland, which secrete a complex digestive fluid. Overall, the pH of the stomach is between 1.5 and 2.5. These gastric glands are made up of different types of cells. Those in the pyloric antrum secrete several hormones, including the majority of the stimulatory hormone gastrin. The much larger glands of the fundus and body of the stomach produce most of the gastric secretions, derived from parietal cells, chief cells, mucous neck cells, and enteroendocrine cells.

There are temporary conditions and long-term, or chronic, diseases and disorders that affect the digestive system, including the stomach These include heartburn which occurs when acidic digestive juices from the stomach go back up the esophagus. Gastroenteritis, stomach flu, is an infection of the stomach and upper part of the small intestine usually caused by a virus. Ulcers develop on the lining of the esophagus, stomach or small intestine, the most common causes being infection with a Helicobacter pylorus (H. pylori) and long-term use of some anti-inflammatory drugs. Lactose intolerance occurs in those who are unable to digest lactose, the sugar primarily found in milk and dairy products. As with all parts of the GI tract, the stomach is susceptible to cancer.

One aspect of gastric reconstruction that is unrelated to stomach diseases is the of bariatric surgery, or stomach by-pass in the treatment of obesity[103]; these techniques are covered later.

[103] Spirou D, Raman J and Smith E, Psychological outcomes following surgical and endoscopic bariatric procedures, *Obesity Reviews*, 2020, 21(6):e12998. doi:10.1111/obr.12998.

An additional factor that should not be ignored related to 'gut feelings'. The Belgian alchemist and physician Jan Baptist van Helmont recorded an experience, early in the 17th century where "*I felt that I did understand, conceive, savor or imagine nothing in the head but rather that I understood and imagined in the midrifts*". He interpreted this as a 'gut feeling', a powerful sense that emotions, perceptions and identity were inextricably associated with the digestive tract. He argued that the stomach was much more than a food processing organ; it was the seat of the mediator between the physical and spiritual realms of the body, indeed the sensitive soul. He was a pioneer in the study of digestion, and well respected; he was the first to suggest that there are chemical agents inside the body which bring about the fermentation and convert food in the stomach into usable energy. However, he was unable to convince the scientific and medical communities about the stomach as the soul, and his thoughts on gut feelings. He was ultimately forced to admit that the sensations he was attempting to describe "*cannot by any words be expressed*". The scientific world has not accepted that the soul is located in the stomach, and is, in fact split into thoughts that the soul is immaterial and imperishable and those that hold that it is material and located in a specific position, but with the brain and heart not the stomach as likely contenders[104]. The medical literature is replete with papers that have 'gut feeling' in the title, but virtually all of them use this expression to catch the eye of the editor and do nothing to clarify any facts about the subject. The one area where there is some genuine connectivity is that which related to the gut microbiota and its relation to brain function and psychiatry[105].

2.2.2.7.3 Small intestine

The small intestine has three segments, the duodenum, jejunum, and ileum. It is a muscular tube that enzymatically breaks down food, using peristalsis to moving food along, mixing it with digestive juices from the pancreas and liver. Contents of the small intestine start in a semi-solid state and end in a liquid form, water, bile, enzymes and mucus causing this change in consistency. The duodenum is shaped like the letter C. The small section at the top, the cap, connects to the pylorus and the liver; this part contains important blood and digestive elements including the hepatic artery, the portal vein, and the common bile duct. From there the duodenum curves around the head of the pancreas, and into the jejunum. A suspensory muscle of the duodenum contracts, widening the angle of the flexure and allowing the contents to move into the jejunum. The duodenum is complex and requires a consistent supply of blood and contains highly sensitive aspects of the nervous system in order to respond to the constantly changing flow of food and fluid through the digestive tract. The lymphatic system is also connected to the duodenum which receives and drains lymphatic fluids, allowing for the removal of toxins from the system. Close to the duodenum is the gallbladder which stores and concentrates bile from the liver before releasing it into the duodenum.

2.2.2.7.4 Large intestine

The large intestine, or colon, a 6-foot long muscular tube that connects the small intestine to the rectum is responsible for processing waste so that emptying the bowels is easy and convenient. It contains the cecum, the ascending, transverse, descending and sigmoid colons. Stool, or waste left over from the digestive process, passes through the colon by peristalsis, ultimately in a solid form, as water is removed. It is stored in the sigmoid colon until it empties it into the rectum once or twice a day.

[104] Santoro G, Wood MD, Merlo L *et al*, The anatomic location of the soul from the heart, through the brain, to the whole body and beyond: A journey through Western history, science and philosophy, *Neurosurgery,* 2009;65(4):633-43. doi:10.1227/01.NEU.0000349750.22332.6A.

[105] Ho P and Ross DA, More than a gut feeling: the implications of the gut microbiota in psychiatry, *Biological Psychiatry*, 2017;81:e35-e37, doi:10.1016/j.biopsycch.2016.12.018.

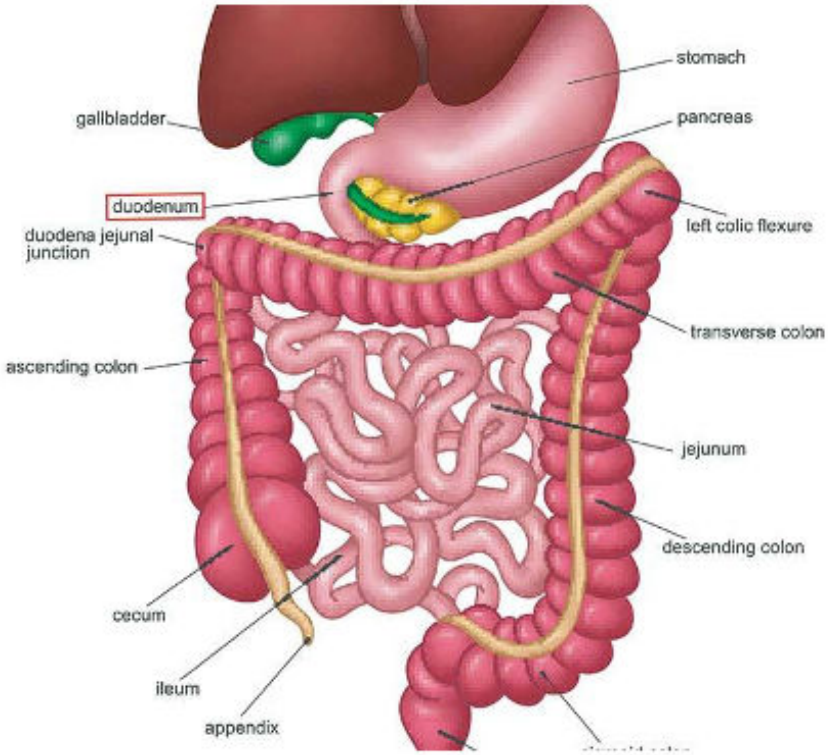

Figure 2.40. The intestines.

2.2.2.7.5 Rectum

The rectum, a straight, 8-inch chamber that connects the colon to the anus. The rectum's job is to receive stool from the colon; sensors send a message to the brain, which decides if the rectal contents can be released. If they can, the sphincters relax and the rectum contracts, disposing its contents.

2.2.2.7.6 Anus

The anus is the last part of this system, being a 2-inch-long canal consisting of the pelvic floor muscles and the two anal sphincters (internal and external). The lining of the upper anus is able to detect rectal contents. The anus is surrounded by sphincter muscles that allow control of stool. The pelvic floor muscle creates an angle between the rectum and the anus that stops stool from coming out when it's not supposed to. The internal sphincter is always tight, except when stool enters the rectum.

2.2.2.7.7 Diseases of the lower GI tract

There are several serious conditions that affect the lower GI tract, some of which are encompassed by the disorder known as irritable bowel syndrome (IBS)[106]. This condition affects 5-10% of otherwise healthy individuals at some time in their lives, being characterized by abdominal pain associated with changes in stool form or frequency. Although it is often a consequence of acute enteric infections, it is associated with individuals, especially in young adult women, that have psychological comorbidities. There is usually a disordered communication between the gut and the brain, leading to motility disturbances and visceral hypersensitivity. Treatments primarily involve patient education, especially about diet and life-

[106] Ford AC, Sperber AD, Corsetti M, *et al*, Irritable bowel syndrome, *The Lancet*, 2020;396:1675-88, doi:10.1016/S0140-6736(20)31548-8.

style; there are also several drugs, including central neuromodulators, that can be used in severe cases. There are no indications for reconstructive procedures. Related to IBS is Crohn's disease, although this is chronic inflammatory condition can affect the whole of the GI tract, causing lesions from mouth to anus and also resulting in extraintestinal complications. The prevalence of Crohn's disease is increasing in adults and children. Genetic predispositions to Crohn's disease have been identified. Common presenting symptoms include diarrhea, abdominal pain, rectal bleeding, fever, weight loss, and fatigue. Celiac disease is a serious autoimmune disease that occurs in genetically predisposed people where the ingestion of gluten (a protein found in wheat, rye and barley) leads to damage in the small intestine. These attacks cause damage to the villi, small fingerlike projections that line the small intestine and promote nutrient absorption. When the villi get damaged, nutrients cannot be absorbed properly into the body. Colon cancer is quite prevalent, typically affecting older adults. It usually begins as small, benign, polyps that form on the inside of the colon. These may be small and produce few symptoms. If colon cancer develops, many treatments are available to help control it, including surgery, radiation therapy and drug treatments, such as chemotherapy, targeted therapy and immunotherapy. If the cancer begins in the rectum, it is usually called colorectal cancer.

2.2.2.8 Urological System

The urological system should be, in principle, quite simple. In relation to the removal of liquid waste and excess water, which constitute urine, the kidneys will have done all the hard work, and all that is required is for a drainage system to take that urine to an external port. However, this is not a trivial matter, as humans, as well as most mammals and lower-order species are better served by a regulated passage of urine rather than an uncontrolled constant dripping process. The urological system, distal to the kidneys, involve two ureters, a bladder and a urethra.

2.2.2.8.1 Ureters

The ureters are thin tubes of muscular tissue that connect the kidneys to the bladder. A thick, fibroelastic, lamina propria lies underneath the epithelium, together forming a protective mucosal layer. There are neither submucosal glands nor a submucosa. There are layers of smooth muscle outside the mucosa, two layers in the upper two-thirds of and three layers in the lower third. Urine is squeezed into the bladder by peristalsis. An outer adventitial layer has fibroelastic connective tissue, with blood vessels, lymphatic vessels and nerves, while folds of mucosa help to protect against reflux of urine when the bladder is full. Several conditions can affect the ureters, most commonly resulting from congenital abnormalities or obstructions. There are many different types of congenital or developmental ureter anomalies. Ureters can be duplicated completely or partially, can be in the wrong place, can be deformed, and can end in the wrong place. The trouble these abnormalities bring is directly related to their effect on the flow of urine. As long as urine flows normally through them, and only in one direction, no harm is done. If one of the ureters has a dead end, has a stricture or stenosis, or a leaky ureterovesical valve, infection is the likely result. A ureter may have an ectopic (out-of-place) orifice (opening): it may enter the bladder, or even another structure, where it does not belong and therefore lacks an adequate valve to control reflux. The primary ureter, or a duplicate, may not even reach the bladder, but rather terminate in a dead end. Urine will stagnate there and eventually cause infection. A ureter can be perfectly normal but in the wrong place, such as behind the vena cava. In this case the ureter may be pinched by the vena cava so that flow is hindered. Other abnormal locations may also lead to compression and impaired flow.

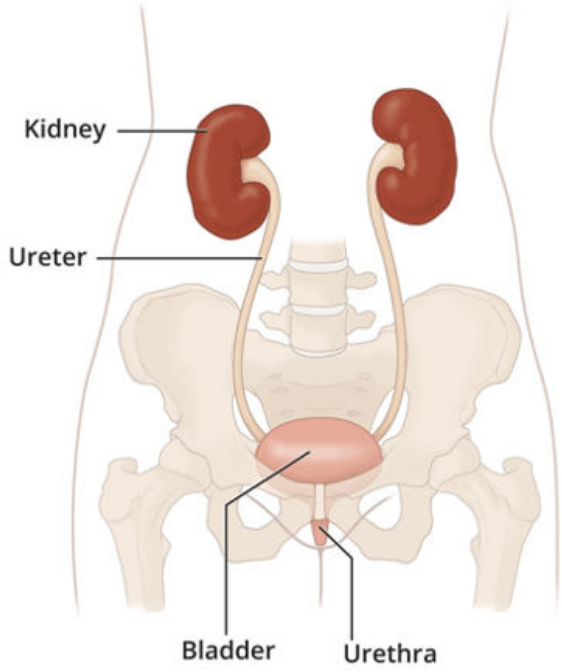

Figure 2.41. The urinary tract.

Ureteral obstructions are fairly common, especially in older men arising from enlarged prostate glands. This can block the flow result in buildup of urine in the bladder. Bladder outflow blockage may also be caused by buildup of pressure in the urinary bladder because of an injury to the nerves or weak muscles, which makes it more difficult for the ureters to empty. Kidney stones may pass into the ureter. An accumulation of urine in the kidneys is called obstructive uropathy, which can lead to hydronephrosis, the swelling of the kidneys. If left untreated, this can cause kidney failure, sepsis and death. Some medications can relieve the effects of obstruction, especially when associated with prostate problems, but interventions are often required. These include inserting a ureteral stent that holds the ureter open or placing a catheter in either the kidney or bladder.

2.2.2.8.2 Bladder

The urinary bladder is a sac that serves as a reservoir for urine. It is located in the extraperitoneal space of the pelvis behind the pubic bones and extends into the abdomen when filled with urine. It has two main, but quite different, parts; the upper part, above the ureteric orifices, is composed of the apex and body, while the lower part is composed of the fundus, trigone, and neck. As the bladder fills, it stretches, simulating afferent signals. Efferent signals result in contraction of the bladder musculature and relaxation of the urethral sphincter, respectively. In addition to mechanoreceptors, various psychological factors like stress, sense of acceptable surroundings, and emotional status play a crucial role in the timing and setting of micturition. The bladder wall has four separate but interacting layers. The inner lining is a mucous membrane of transitional epithelium, which is continuous with the lining of the ureters. When the bladder is empty, numerous folds, the rugae, can be seen; these allow the bladder to expand as it fills with urine. The submucosa, composed of connective tissue with elastic fibers, supports the mucous membrane. The muscularis, composed of smooth muscle, has interwoven, multidirectional fibers, collectively referred to as the detrusor muscle, contraction of which expels urine from the bladder. On the superior surface of the bladder is the parietal peritoneum, elsewhere the outer later being fibrous connective tissue. At the floor

of the bladder is a triangular area called the trigone, which has two openings from the ureters and one into the urethra, the latter opening being encircled by the internal urethral sphincter.

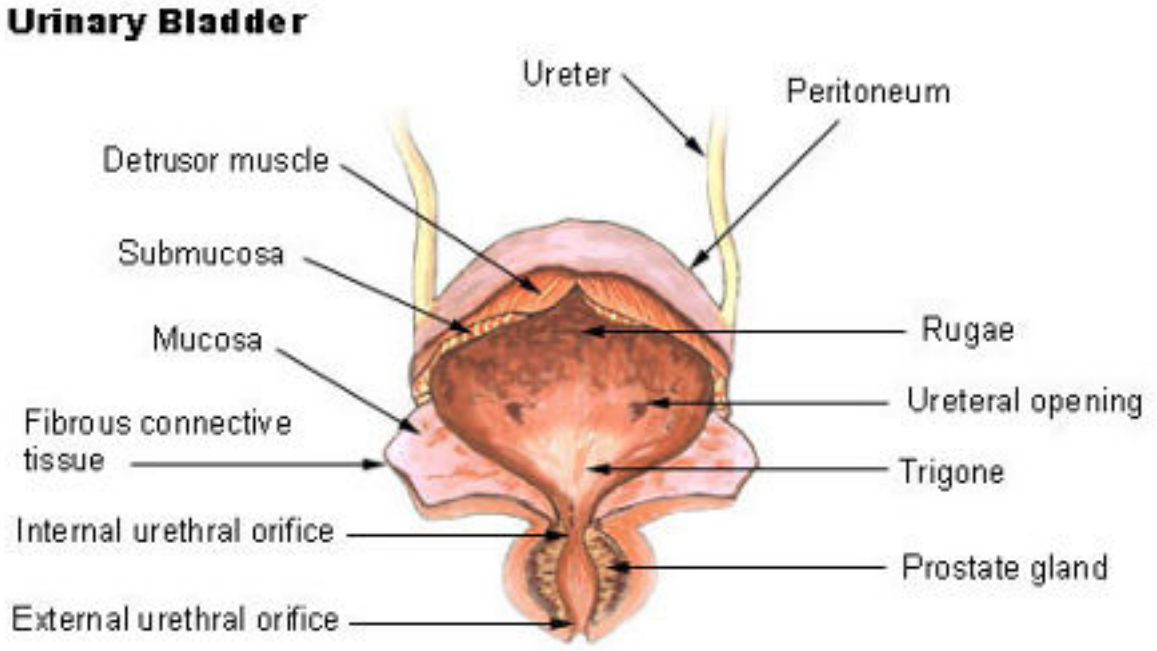

Figure 2.42. The urinary bladder of the human male.

As noted at the beginning of this section, the function of the urological system should, in principle, be quite simple, and it would seem obvious that the functional center of the control of urine flow would be the bladder. It is necessary, however, to look into this a little more closely. That this is not a simple matter is seen from the fact that, a decade ago, it was estimated that over 45% of the world population aged over 20 (i.e., close to 2 billion people) are affected by lower urinary tract symptoms[107]. An unhealthy bladder may be defined as one that is associated with increased voiding frequency or cancer, symptoms including the overactive bladder, bladder outlet obstruction, bladder pain syndrome, interstitial cystitis, urinary tract infection and bladder cancer. These conditions are divided into three categories, storage, voiding and post-micturation, and symptoms include urinary incontinence (including stress and urgency), increased daytime voiding frequency, nocturia, hesitancy and incomplete emptying. Although it might seem intuitively obvious that the control of urine flow out of the bladder is primarily a biomechanical / hydrodynamic characteristic or the urethral sphincter, the real control is vested in the signaling pathways in the urothelium, and especially in the lamina propria[108,109].

[107] Lukacz ES, Sampselle C, Gray M, *et al*, A healthy bladder: a consensus statement, *International Journal of Clinical Practice,* 2011;65:1026-36, doi:10.111/j.1742-1241.2011.02763.x.
[108] Birder L and Andersson K-E, Urothelial signaling, *Physiological Reviews,* 2013;99:653-80, doi:10.1152/physrev.000330.2012.
[109] Andersson K-E and McCloskey KD, Lamina Propria: The functional center of the bladder? *Neurourology and Urodynamics,* 2014; 33:9–16, doi;10.1002/nau.22465.

As described by Arya and Weissbart[110], the urinary bladder and urethral sphincters are ultimately controlled by the brain through a complex network of neurons that allow urine to be stored and voided when socially appropriate. The storage is assisted by distension of the bladder during filling, synchronized with contraction of the sphincters. The bladder is supplied by the autonomic nervous system (both sympathetic and parasympathetic) and somatic nerves, which are all under central control. The sympathetic nerves originate at the T10-L2 segment of the spinal cord, relay through the superior hypogastric plexus and terminate on the detrusor muscle and internal urethral sphincter. The parasympathetic nerve originates in S2-S4 segments and terminate on the cholinergic muscarinic receptors on the bladder, while the somatic nerves, also originating at S2-S4 levels terminate on the nicotinic receptors of the external urethral sphincter. Thus, there is a complex network of nerves that control bladder function, replication of which during any attempted reconstruction procedure is very difficult.

2.2.2.8.3 Congenital bladder defects

Congenital urinary bladder anomalies may cause infection, retention, incontinence, and reflux. Symptomatic anomalies may require surgery. The most common conditions are as follows:

A bladder diverticulum is a herniation of the mucosa through a defect in the muscle. It predisposes to urinary tract infection and may coexist with vesicoureteral reflux. With bladder exstrophy, there is a failure of midline closure from the umbilicus to the perineum, resulting in continuity of the mucosa with abdominal skin, separation of the pubic symphysis, and bifid genitalia. The bladder is open suprapubically so that urine drips from the open bladder rather than through the urethra. The bladder can usually be reconstructed and returned to the pelvis; reconstruction of the genitals is often required. A neurogenic bladder is an abnormality caused by neurologic disorders, including spinal cord or central nervous system abnormalities, trauma, or the sequelae of pelvic surgery. The bladder may be flaccid, spastic, or a combination. A flaccid bladder has high-volume, low-pressure, and minimal contractions. A spastic bladder has normal or low-volume, high-pressure, and involuntary contractions. When present, chronically elevated bladder pressure often causes progressive kidney damage. The underlying neurologic abnormality is usually readily apparent. Treatment goals include lowering risk of infection, maintaining adequate bladder storage pressure and volume, effective bladder emptying, and achieving social continence.

2.2.2.8.4 Bladder dysfunction and urinary incontinence

Although the mechanisms of urinary flow control appear simple in principle, there is much that can go wrong with the bladder. This is compounded by the influence of several co-morbidities on bladder function, for example with Parkinson's disease[111] and multiple sclerosis[112], and by the enormous sociological implications of poor bladder control, especially in women[113], including serious effects on sexual health[114]. There are many facets to the bladder dysfunction syndrome, with interconnectivity

[110] Arya NG and Weissbart SJ, Central control of micturition in women: Braid-bladder pathways in continence and urgency urinary incontinence, *Clinical Anatom*y, 2017;30:373-84. doi:10.1002/ca.22840.
[111] Sakakibara R, Panicker J, Finazzi-Agro E *et al*, A guideline for the management of bladder dysfunction in Parkinson's disease and other gait disorders, *Neurourology and Urodynamics,* 2016; 35:551–63. doi;10.1002/nau.22764.
[112] Wintner A, Kim M, Bechis S, *et al,* Voiding dysfunction in multiple sclerosis, *Seminars in Neurology*, 2016;36(1):212-8. doi:10.1055/s-0035-1571212.
[113] Vo A and Kielb SJ, Female voiding dysfunction and urinary incontinence, *Medical Clinics of North America,* 2018;102:313–24. doi:10.1016/j.mcna.2017.10.006.
[114] Duralde ER and Rowen TS, Urinary incontinence and associated female sexual dysfunction, *Sexual Medicine Reviews* 2017;5:470e485. doi:10.1016/j.sxmr.2017.07.001.

between several symptoms. As implied by Hanno *et al,* there are disagreements over definitions and characterization of the different compartments of the syndrome[115]. The one overarching feature of dysfunction and pain is interstitial cystitis; the causes and mechanisms are unclear, but features can include granulation tissue in the lamina propria, mast cell accumulation and lymphocyte activation. The overactive bladder, while not a disease *per se*, constitutes a symptom complex characterized by urgency, frequency and nocturia[116]. When the condition is refractory (i.e., it does not respond to any treatment), it may include occult neurogenic bladder, outlet obstruction, urothelial dysfunction, ischemia, inflammation, and autonomic dysfunction.

A further important factor in the development of aging bladder dysfunction is the variation in blood flow[117]. Chronic ischemia of the bladder (i.e., where there is insufficient blood supply to the bladder), likely caused by bladder outflow obstruction or atherosclerosis, may be associated with oxidative stress, and the expression of tissue damaging molecules in the bladder wall, producing detrusor overactivity and / or underactivity and the inability to empty the bladder[118].

Although, as already mentioned, there are many potential causes of urinary incontinence, a prevailing mode is that of stress urinary incontinence. Again, there could be several contributing factors but at the core of the problem is 'pure' or genuine' stress incontinence, which is the condition in which increments of intra-abdominal pressure produce involuntary urine loss. The condition occurs as a result of urethral sphincteric failure in the absence of involuntary detrusor contractility[119]. This will be seen as an important factor in treatment of incontinence by reconstructive procedures later in the book. This also emphasizes the significant differences seen between males and females in relation to bladder dysfunction and, especially urinary incontinence, the latter being especially prevalent in women.

2.2.2.8.5 Prostate gland

In the context of the last sentence in the above paragraph, the most important aspect of bladder dysfunction in males centers on the role of the prostate gland. This is part of the male reproductive system and is discussed within that section later; it is briefly mentioned here because it its role in urinary tract problems. It is located in front of the rectum and just below the bladder. It is usually described as being the size of a chestnut and conical in shape, with a base and an apex, and anterior, posterior and lateral surfaces. Its function is to produce fluid for the semen that transports sperm. There are three conditions that can affect the prostate, hyperplasia, cancer and prostatitis. The first of these, specifically benign prostatic hyperplasia (BPH) is most relevant here, This is an increase in the size of the prostate, without the presence of malignancy, being quite prevalent in old age. The enlarged prostate may compress the urethra leading to impaired storage of urine and symptoms of frequency, urgency, nocturia, difficulty of initiating micturition, poor or intermittent urine stream and terminal dribbling. It is due to hyperplasia of the stromal and glandular epithelium of the transition zone of the prostate[120].

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[115] Hanno P, Fall M, Meijink J *et al,* Towards a new paradigm in bladder pain syndrome and interstitial cystitis, *British Journal of Urology Internationa*l, 2020;126:549-50. doi:10.111/jbju.15223.

[116] Chen L-C and Kuo H-C, Pathophysiology of refractory overactive bladder, *Lower Urinary Tract symptoms,* 2019;11:177-81. doi:10.111/luts.12262.

[117] Andersson K-E, Boedtkjer CB and Forman A, The link between vascular dysfunction, bladder ischemia and ageing bladder dysfunction, *Therapeutic Advances in Urology*, 2017;9(1):11-27, doi:10.1177/1756287216675778.

[118] Yamaguchi O, Nomiya M and Anderssom K-E, Functional consequences of chronic bladder ischemia, *Neurourology and Urodynamics,* 2014; 33:54–8. doi;10.1002/nau.22517.

[119] Rubilotta E, Balzano M, D'Amico A, *et al*, Pure stress urinary incontinence: analysis of prevalence, estimation of costs, and financial impact, *Biomed Central Urology*, 2019;19:44. doi/10.1086/s12894-019-0468-2.

[120] Aaron L, Franco O and Hayward SW, Review of prostate anatomy and embryology and the etiology of BPH, *Urological Clinics of North America*, 2016;43(3):279-88. doi:10.1016/j.ucl.2016.04.012.

2.2.2.8.6 Urethra

The urethra is positioned between the bladder and the urinary meatus. Internal and external urethral sphincter muscles control micturition. In females, the main functions are the transportation of urine out of the body without any reflux, and protection against pathogenic bacteria. In males, there is an added function – the expulsion of sperm. In both genders, the lumen is surrounded by a layer of stratified columnar epithelium, which is protected from the highly acidic environment by mucus, which keeps the urethra moist and supple. There is then a mucus-secreting submucosa, surrounded by an inner longitudinal muscle and an outer circular muscle, which combine to provides strong contraction power. The internal urethral sphincter is composed of smooth muscle fibers, a continuation of the bladder detrusor muscle**.** The external sphincter is kept tight around the urethra through the involuntary control of the sympathetic nervous system via excitatory receptors in the urethra and bladder neck. Before micturition, the parasympathetic nerves relax the internal sphincter through the release of acetylcholine, while other neurotransmitters excite the detrusor muscle, causing the bladder to contract.

The female urethra is only about 4 cm long, ending just above the vaginal opening and below the clitoris. It has three parts, the internal sphincter, the urethrovaginal muscle, and the external sphincter, often called the compressor muscle as the urethra is kept compressed against the vagina in the absence of micturition. Arterial blood supply is provided by the internal pudendal and vaginal arteries. At the end of the female urethra, two mucous glands secrete lubricants, providing an additional protective barrier against infection. Childbirth may damage the muscles of the pelvic floor and pelvic ligaments and the pudendal nerve, both of which may cause incontinence. The male urethra is around 22 cm in length and is also comprised of three segments. The first section is the prostatic urethra; ejaculatory ducts containing a mixture of semen produced by the testes, alkaline fluid produced by the prostatic ducts, and seminal fluid empty into this section. The membranous urethra travels through the external sphincter and the pelvic floor and deep perineal pouch. This part of the urethra is narrow and less supple and so it is more likely to get blocked by stones. The penile urethra travels through the center of the penis, through the bulb and corpus spongiosum, and terminates at the meatus or external urethral orifice.

Urethra pain is a common symptom since the entire urinary system is extremely well innervated. Inflammation, infection, spasm, stenosis, or blockage can cause significant urethral discomfort. Urolithiasis, the partial or complete blockage of the urethra by crystallized minerals, is a common occurrence, associated with low water intake combined with high salt levels. Urinary tract infections are primarily caused by the transfer of *E. Coli* bacteria from the area around the anus into the distal opening of the urethra. Due to the shorter length of the urethra in females, cystitis or infection of the bladder is much more common than in males. Lower UTIs include urethritis and cystitis. Prostatitis is also included within the group of lower urinary tract infections. As already mentioned, most of these pathologies are caused by the transmission of bacteria from the anus. In lower UTIs, urethra disorder treatments usually involve antibiotics when caused by bacterial infections. Urethral stenosis or urethral stricture – a narrowing of the lumen caused by swelling, scar tissue or congenital abnormalities – may require dilation. Dilation involves the insertion of gradually larger wires until the lumen is wide enough to allow the passage of urine**.**

Urethroplasty is the removal of a section of the urethra or its enlargement using other tissues such as skin. This can permanently solve a case of urethral stenosis. A urethrotomy is a surgical procedure where the urethra is incised**,** loosening the stricture. Small hollow tubes, or stents, can be inserted into the urethra to temporarily relieve blockages before surgery or other treatment of the cause.

2.2.2.9 Respiratory System

The respiratory system has two major divisions: the upper and lower respiratory tracts. In addition to the structures contained within these tracts, certain muscles of the thorax are also involved in respiration; the most important being the muscular diaphragm, which lies below the lungs and separates the thorax from the abdomen. The upper tract is involved in the conduction or the movement of air into and out of the body. However, no gas exchange occurs in these areas. The nasal cavity is a large, air-filled space in the skull above and behind the nose, being a continuation of the two nostrils. As inhaled air flows through the nasal cavity it is warmed and humidified. Hairs in the nose help trap larger foreign particles in the air. In addition to its respiratory functions, the nasal cavity also contains chemoreceptors that are needed for the sense of smell and that contribute importantly to the sense of taste. The pharynx is a tube-like structure that connects the nasal cavity and the back of the mouth to other structures lower in the throat, including the larynx. The pharynx has dual functions: both air and food pass through it, so it is part of both the respiratory and digestive systems. The larynx connects the pharynx and trachea and helps to conduct air through the respiratory tract. It also contains the vocal cords, which vibrate when air flows over them, thereby producing sound. Certain muscles in the larynx move the vocal cords apart to allow breathing. Other muscles in the larynx move the vocal cords together to allow the production of vocal sounds. The latter muscles also control the pitch of sounds and help control their volume. A very important function of the larynx is protecting the trachea from aspirated food. When swallowing occurs, the backward motion of the tongue forces the epiglottis to close over the entrance to the larynx. If swallowed material does start to enter the larynx, it irritates the larynx and stimulates a strong cough reflex This generally expels the material out of the larynx and into the throat.

The trachea and other passages of the lower respiratory tract conduct air to the lungs. These passages form an inverted tree-like shape with repeated branching as they move deeper into the lungs. There are 1,500 miles of airways conducting air through the human respiratory tract. The trachea is the widest passageway in the respiratory tract, about 2.5 cm wide and 10-15 cm long. It is formed by rings of cartilage, giving strength and resilience. The trachea branches at the bottom to form two bronchial tubes, the right and left bronchi. Each of these branches into smaller, secondary bronchi; and still smaller tertiary bronchi. The smallest bronchi branch into tubules called bronchioles, which end in alveolar ducts, terminating in clusters of minuscule air sacs, the alveoli, in the lungs. The structure and function of the lungs have been discussed in the earlier section on major organs.

2.2.2.9.1 Disorders of the respiratory system

Many of the disorders of the lung can also affect other structures in the system, especially in the lower part of the tract. The trachea provides a good example, which exhibits two main types of disorder. Tracheal stenosis is a narrowing that prevents air from fully reaching the lungs. In the most severe cases, the patient may be dependent on a tracheotomy tube for breathing. Tracheomalacia is characterized by cartilage in the walls of the trachea that has broken down, causing weakness or floppiness. Infants may be born with tracheomalacia, but adults can also acquire it in later life. Tracheal tumors are rare but can also cause narrowing of the structure and coughing of blood. Tumors may be benign**,** such as pleomorphic adenoma, and squamous cell papilloma, or malignant**,** such as squamous cell carcinoma.

If the vocal cords become inflamed, develop growths, or become paralyzed, they may cause a voice disorder. Common voice disorders include laryngitis, dysphonia, noncancerous lesions such as polyps or cysts, precancerous and cancerous lesions, and vocal cord paralysis. Risk factors include aging, allergies, GERD, neurological disorders, smoking, dehydration, thyroid dysfunction and voice over- or mis-use.

The vocal cords have a disproportionate influence on personality - such a small anatomical feature controls vocal and sound interactions with others that cannot be achieved in any other way. However,

they have very limited connectivity with spirituality, where (as discussed previously), hearing voices dominate over the delivery of voices. A very robust analysis of 'hearing spiritually significant voices'[121] almost exclusively emphasizes the spirituality of hearing, or voice hearing, not voices themselves. Interestingly, the Chakra system described in ancient Sanskrit, and followed in modern day yoga, includes the throat chakra, or Vishudda, which governs the energy associated with communication (and truth and self-expression), but which involves the whole of the anatomic region of the jaw, neck and larynx, including vocal cords along with many other tissues. At a more mundane, but practical level, William Faulkner, who is difficult to understand in many of his writings, identified the profound impact of the vocal cords when describing an encounter between some US navy personnel and some women in a dockside bar in Naples, Italy (Faulkner's punctuation being maintained):

> *Now and then they spoke to one another in Italian. The women in Italian, the men in English, as if language might be the sex difference, the functioning of the vocal cords the inner biding until the dark pairing time. The men in English, the women in Italian: a decorum as of two parallel streams separated by a levee for a little while.*
> **William Faulkner, *Divorce in Naples***[122]

2.2.2.10 Reproductive Systems

I refrained from quoting or illustrating any artistic or spiritual representational aspects of some of the above sections since there aren't too many tasteful and educational characteristics of either the GI or urinary systems. There are, of course, many references to the reproductive system in such discourse, but very few of them qualify as both tasteful and educational, so that I extend that restraint here. As noted previously, I do not discuss reproduction itself in this book on reconstruction, but a few points have to be made in the context of anatomical and physiological characteristics of the tissues of the reproductive system in case they need reconstruction.

2.2.2.10.1 Male reproductive system

The male reproductive system includes the penis, scrotum, testes, epididymis, vas deferens seminal vesicles, prostate and urethra; the latter two have been discussed in the section on the urinary system because the urethra is also part of that system, and the prostate is involved in some aspects of its dysfunction. The penis could also be considered as part of the urinary system, but its prime function relates to reproduction. The penis consists of the root (which is attached to the lower abdominal structures and pelvic bones), the visible part of the shaft, and the glans penis (the cone-shaped end). The opening of the urethra (the channel that transports semen and urine) is located at the tip of the glans penis. The base of the glans penis is called the corona. In uncircumcised males, the foreskin (prepuce) extends from the corona to cover the glans penis. The penis includes three cylindrical blood-filled sinuses of erectile tissue. The two larger ones, the corpora cavernosa, lie side by side. The third sinus, the corpus spongiosum, surrounds most of the urethra. When these spaces fill with blood, the penis becomes large, rigid and erect.

[121] Cook CCH, Powell A, Alderson-Day B *et al*, Hearing spiritually significant voices: A phenomenological survey and taxonomy, *Medical Humanities*, 2020;0:1-12, doi:10.1036/medhum-2020-012021.
[122] William Faulkner, 'Divorce in Naples' in Collected Stories, p 877, Vintage International, New York, 1995, ISBN 0-079-76403-8.

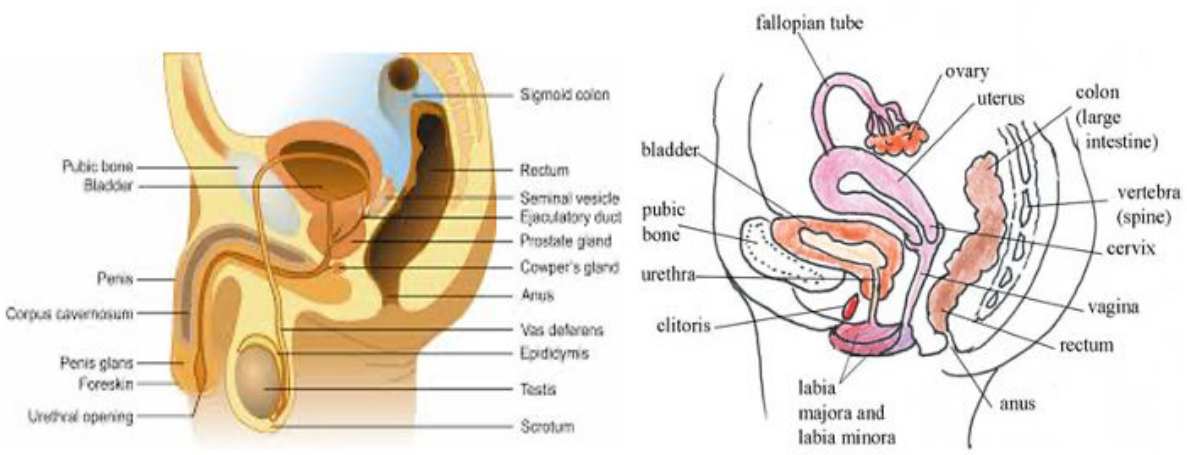

Figure 2.43. Male (left) and female (right) reproductive systems

The scrotum is a sac that surrounds and protects the testes, also acting as a temperature-control system since the testes need to be slightly cooler for normal sperm development. The cremaster muscles in the wall of the scrotum relax to allow the testes to hang farther from the body to cool or contract to pull the testes closer to the body for warmth or protection. The testes are oval bodies that average about 4 to 7 cms in length. The testes have two primary functions, producing sperm and testosterone. The epididymis consists of a single coiled microscopic tube that collects sperm from the testis allowing it mature and acquire the ability to move through the female reproductive system and fertilize an ovum. One epididymis lies against each testis. The vas deferens is a firm tube that transports sperm from the epididymis. One duct travels from each epididymis to the back of the prostate and joins with one of the two seminal vesicles, which are located above the prostate and join with the vas deferens to form the ejaculatory ducts, which travel through the prostate.

2.2.2.10.2 Female reproductive system

Within the female reproductive system, ovaries produce the eggs, known as ova or oocytes, which are then transported to the fallopian tube where fertilization by a sperm may occur. The fertilized egg then moves to the uterus, where the uterine lining has thickened in response to the normal hormones of the reproductive cycle. Once in the uterus, the fertilized egg can implant into thickened uterine lining and continue to develop. If implantation does not take place, the uterine lining is shed as menstrual flow. During menopause, the female reproductive system gradually stops making the female hormones necessary for the reproductive cycle to work.

The female reproductive anatomy includes both external and internal structures. The main external structures include the labia majora which encloses and protects the other external reproductive organs and the labia minora, which lie just inside the labia majora, and surround the openings to the vagina and urethra. This skin here is very delicate is easily irritated and swollen. The two labia minora meet at the clitoris, a small, sensitive protrusion which is covered by a fold of skin, called the prepuce; the clitoris is very sensitive to stimulation and can become erect.

The internal reproductive organs include the vagina, a canal that joins the cervix (the lower part of uterus) to the outside of the body. The uterus is a hollow, pear-shaped organ that is divided into two parts, the lower cervix and the main body, called the corpus. The corpus can easily expand to hold a developing baby. The ovaries are small, oval-shaped glands, located on either side of the uterus, which produce eggs

and hormones. The Fallopian tubes are narrow tubes that are attached to the upper part of the uterus and serve as pathways for the ova (egg cells) to travel from the ovaries to the uterus.

2.2.2.10.3 Congenital conditions of the reproductive systems

Congenital abnormalities of the male reproductive system usually concern variations in the presence or form of the testes or penis. Concerning the testes, anorchia refers to being born without any testes, microorchidism and macrorchidism involve abnormally small or large testes respectively and cryptorchidism is the absence of one or both testes from the scrotum, that is undescended testes, which is relatively common. Polyorchidism is the presence of more than two testes. Hypospadias is a variation in fetal development of the penis where the urethra does not open from its usual location in the head of the penis. An epispadias is a rare condition in which the urethra ends in an opening on the upper aspect of the penis. Penile torsion is quite common, the penis appearing rotated on its axis. Congenital absence of the vas deferens is a condition in which the vasa deferentia fail to form properly, either be unilaterally or bilaterally.

In females[123], three situations can arise with the vulva which may require reconstruction; patients with congenital adrenal hyperplasia (CAH), patients with late-onset CAH in whom clitoromegaly may result, and patients with androgen-secreting tumors of the ovary. With CAH, the objective of reconstructive procedures is usually to delay the surgery until the anomalous structures have reached a size that permits effective surgery, and but before the abnormalities present become seriously embarrassing. Most vulvo-vaginal surgery attempts to create an anatomically acceptable and functional vulva and vagina, which will grow with the child through adolescence into adulthood. Clitoromegaly is an abnormal enlargement of the clitoris; the original techniques involved amputation of the clitoris, leaving a non-functioning clitoral stump but today clitoral recession with retention of the clitoris in its entirety is normally considered better.

Congenital malformations of the vagina fall into three categories: remnant cysts, obstructive outflow tract disorders and congenital absence of the vagina. Vaginal agenesis is a rare disorder that occurs when the vagina does not develop, and the uterus may only develop partially or not at all. This condition is present before birth, and may also be associated with kidney, heart, or skeletal abnormalities. The management of these patients falls is based on two factors, the need to be sexually active, and the psychological impact of the knowledge that these individuals have no vagina. Non-surgical management agenesis may involve requires dilatation of the vaginal dimple while surgical management (neovaginoplasty) includes attempts to create a functional vagina; for example, by using peritoneum or skin flaps.

2.2.2.10.4 Diseases, dysfunction and trauma of the reproductive systems

Dysfunction of the reproductive systems is a topic of immense sociological significance at this time, but only of marginal relevance to reconstruction of the body, In an excellent review of male reproductive disorders a few years ago[124], Skakkebaek *et al* discussed the decreases in populations, worldwide, and an association with male reproductive problems, including some of the congenital disorders mentioned above, but with an emphasis on disorders of sex development, low testosterone levels and poor semen quality. Although these conditions may arise from genetic mutations, the evidence would suggest that it is more related to environmental exposure of fetal testes, with endocrine disruptors such as phthalates being a main focus. With females, the most common troublesome conditions are endometriosis, cervical cancer, pelvic inflammatory disease, interstitial cystitis, ovarian insufficiency, obesity and polycystic ovary syndrome. For both genders, sexually transmitted diseases, including HIV / Aids are significant factors.

[123] Edmonds DK, Congenital malformations of the genital tract and their management, *Best Practice & Research Clinical Obstetrics & Gynaecology*, 2003; 17(1):19-40. doi:10.1053/ybeog.2003.0356,

[124] Skakkebaek NE, Meyts ER, Louis GMB, *et al,* Male reproductive disorders and fertility trends; Influences of environment and genetic susceptibility, *Physiological Reviews*, 2016;96;55-97, doi:10.1152/physrev.00017.2015.

Trauma occurs not infrequently to the reproductive systems, along with the closely allied urinary tract; military combat, especially involving improvised explosive devices[125] and sexual violence[126] are major factors. These injuries tend to be individual in character and subsequent management. They will be dealt with in appropriate places with respect to reconstruction technologies.

2.2.2.11 Soft Connective Tissues, Adipose Tissue, Skin and Associated Structures

Leaving aside the major tissues, organs and systems covered in the previous parts of this Chapter, there are still many other structures in the body that have a profound effect on function and feature prominently in several reconstructive technologies. Indeed, two of the features for which the background is given here, abdominal hernia and breast reconstruction, are among the most significant, and most controversial, aspects of the technologies discussed in this book.

2.2.2.11.1 Fascia

Fascia is composed of sheets of connective tissue that are seen in the body encasing organs, muscles, and bones, which separate, stabilize, and impart strength to these structures. Not surprisingly with a rather vague definition there have been many arguments about the limits of what tissues may be considered as fasciae, but generally they are classified as superficial, deep, visceral, or parietal, with sub-categories within these classes.

Superficial fascia occurs directly under the skin and superficial adipose layers and contains membranous layers with loosely packed interwoven collagen and elastic fibers. It is thicker in the trunk than in the limbs and becomes thinner peripherally. These layers may include muscle fibers, such as the platysma muscle in the neck, the external anal sphincter, and the dartos fascia in the scrotum.

Deep fascia surrounds bones, muscles, nerves, and blood vessels, having a fibrous consistency and being rich in hyaluronan. It is highly vascularized and contain well developed lymphatic channels. There are 2 subtypes: aponeurotic fascia are sheets of pearly-white fibrous tissue that attach muscles needing a wide area of attachment such as the fascia of limbs, thoracolumbar fascia, and rectus sheath. Epimysial fascia, or the epimysium, is the connective tissue sheath that surrounds skeletal muscle and can, in some cases, connect directly to the periosteum of bones. In many areas, superficial and deep fascial layers are connected by fibrous septa, creating a connection network that incorporates fat lobules and makes up the deep adipose tissue layer.

Visceral fascia surrounds organs in cavities such as the lung (pleura), and heart (pericardium). Parietal fascia describes tissues that line the wall of a body cavity just outside of the parietal layer of serosa.

Fascia is one of the richest sensory organs in the body, being embedded in nerve endings and mechanoreceptors. It plays a major role in the perception of posture and movement, affecting proprioception and coordination.

There are several general causes of fascial dysfunction, including trauma, sub-optimal nutrition and habitual postures and lack of mobility, which can impact on the fascia's ability to glide and slide, inhibiting the distribution and transmission of tension across the body. Fascial dysfunction is associated with pain, stiffness, tissue fatigue and reduced performance and function. Compartment

[125] Banti M, Walter J, Hudak S *et al*, Improvised explosive device-related lower genitourinary trauma in current overseas combat operations, *Journal of Trauma and Acute Care Surgery*, 2015;80(1):131-4, doi:10.1097/TA0000000000000864.

[126] Chisholm CA, Bullock L and Ferguson JE, Intimate partner violence and pregnancy: epidemiology and impact, *American Journal of Obstetrics and Gynecology*, 2017; 217(2(:141-3, doi:10.1016/j,ajog.2017.05.042

syndrome is a condition where the tissues within a fascial layer experience higher than normal pressures leading to pain, pallor, loss of pulses, and eventual paresthesia. It can develop in morbidly obese patients or following crush injuries, severe burns, and impact injuries. In patients who develop compartment syndrome, treatment regularly involves a fasciotomy, a procedure where the compressing fascia is cut to relieve compartment pressure and allow the muscle to swell, decrease pressure, and restore blood flow and nerve function. Plantar fasciitis is a common condition that results in inferior heel pain. Necrotizing fasciitis is rare but sometimes fatal soft tissue infection associated with inflammation and necrosis of multiple layers, including fascia, muscle, subcutaneous fat and overlying skin.

2.2.2.11.2 Adipose tissues

While prokaryotes and single-celled eukaryotes store lipids in intracellular organelles known as lipid droplets, multicellular organisms developed specialized cells to store them. In mammals, two principal types of lipid-storing adipose tissue exist, white (WAT) and brown (BAT)[127]. BAT develops embryonically, derived from precursor cells in the mesoderm that also give rise to skeletal muscle cells and a portion of white adipocytes.

Brown adipocytes contain multilocular lipid droplets and high numbers of mitochondria, and primarily function to dissipate stored energy in the form of heat. The majority of adipose tissue in mammals is WAT, which is primarily composed of large adipocytes that contain a single lipid droplet and markedly fewer mitochondria than brown adipocytes. The principal function of WAT is that of controlling energy homeostasis *via* the storage and release of lipids in response to systemic nutritional and metabolic needs. It is distributed throughout the body in distinct depots which include visceral depots such as omental, mesenteric, retroperitoneal, gonadal, and pericardial WAT These are commonly associated with metabolic disorders, such as diabetes and cardiovascular disease. Subcutaneous WAT is located under the skin and, in some areas of the upper abdomen and lower, gluteofemoral regions. In addition, discrete tissue-associated adipose depots are broadly distributed across the body, often small in size, intricate in microanatomy, closely associated with other anatomic structures, and perform tissue- and organ-specific functions. There are some prominent tissue-associated adipose depots in different anatomic locations, including the dermis, certain craniofacial tissues, mammary tissues and mesenteric tissues.

The skin consists of consecutive layers of stratified epidermis, fibroblast-rich dermis, and dermal WAT, which contains mature, unilocular white adipocytes. Skin appendages, principally hair follicles, traverse through multiple layers come in close contact with the WAT.

Craniofacial structures have a close association with several adipose pads, and WAT significantly affects facial recognition and age perception. Adipocytes in the face are partly derived from the neural crest which explains why some types of congenital WAT dystrophies, specifically congenital infiltrating lipomatosis, are restricted to the face. Facial WAT consists of deep (including sub-orbital, retro-orbital, and buccal adipose pads) and superficial depots, which are numerous and distribute broadly under the facial skin. In normal adults, facial WAT significantly reduces with age, contributes to facial aging and the loss of adipose volume results in wrinkling of the skin.

In the mammary gland, the milk-producing epithelial ducts are surrounded by mammary adipose tissue, primarily composed of white adipocytes. Compared with other mammals, adipocyte abundance represents a lower percentage of the human breast in favor of more fibrous connective tissue and varies widely between individuals. The mammary gland epithelium undergoes cyclic remodeling consisting of

[127] Zwick RK, Christian F. Guerrero-Juarez CF, Valerie Horsley V*, et al,* Anatomical, physiological, and functional diversity of adipose tissue, *Cell Metabolism*, 2018; *27*: 68-83. doi:10.1016/j.cmet.2017.12.002.

pregnancy, lactation, and involution stages, and the WAT remodels along with it. During pregnancy, mammary adipocytes expand in size and nearly double in lipogenic capacity. However, when lactation initiates, lipogenesis rates within WAT decrease and its area dramatically decreases.

Mesenteric WAT is a distinct depot located in the double fold of peritoneum. It comes into contact with intestines at the site of mesentery attachment; it also plays an important role in the pathogenesis of inflammatory bowel diseases. In particular, a prominent expansion of WAT around the circumference of intestines, so-called ‘‘creeping fat,’’ is a pathological hallmark of such diseases. This expansion largely occurs *via* hyperplasia, as it becomes enriched by many small adipocytes, possibly induced by the increased penetration of bacteria from the intestinal lumen with resulting pro-inflammatory signaling driving disease progression.

2.2.2.11.3 Structures of the abdomen

It is instructive to consider the structure of the abdomen to see the complexity and variety of some of these tissues. The abdomen connects the thorax and pelvis. The abdominal wall is formed of skin, fascia, and muscle and encases the abdominal cavity and viscera, protecting the internal organs by distention, generation of intrabdominal pressure, and moving the vertebral column. The abdomen is relatively deficient in skeletal support, involving only the vertebral column and lower ribs posteriorly. This relative bony deficiency allows flexibility and distensibility to accommodate dynamic changes in the abdominal contents. From superficial to deep zones, the layers of the abdominal wall include skin, subcutaneous tissue, abdominal muscles with their investing fascia and aponeuroses, transversalis fascia and parietal peritoneum.

The sensory function of skin, in generic terms, has already been discussed. Three layers can be identified under the dermis, a superficial adipose layer, a membranous layer and a deep adipose layer. Beneath these layers lies the deep fascia enveloping the abdominal wall muscles.

The abdominal muscles may be considered in relation to anterolateral and posterior components. The former include five paired muscles: the external oblique, internal oblique, transversus abdominis, rectus abdominis, and pyramidalis. Posterior muscles include psoas major and quadratus lumborum bilaterally. The abdominal muscles control movements of the trunk, especially flexion, extension, lateral flexion, and rotation. Simultaneous contraction of these muscles facilitates the generation of intraabdominal and intrathoracic pressure critical in sneezing, coughing, vomiting, and defecating. Anterior chest wall strength and movement are influenced by the external oblique, internal oblique, and transversus abdominis. Anteromedially these layers fuse to form a rectus sheath that encloses the rectus abdominis and pyramidalis muscles. In the midline, the combined aponeuroses of these muscles fuse to form the linea alba.

The posterior abdominal wall muscles include the quadratus lumborum, contraction of which cause lateral flexion and extension of the vertebral column, and depression of the rib cage, and the psoas major which runs inferiorly, joining the iliacus to insert into the lesser trochanter of the femur. Contraction causes flexion of the thigh.

2.2.2.11.4 Hernias

A hernia occurs when an internal organ or other structure protrudes through a muscular wall that normally contains it. Most occur within the abdominal cavity. Inguinal hernias are the most common, being more prevalent in men; the inguinal canal is a passageway for the spermatic cord and blood vessels leading to the testes. In women, the inguinal canal contains the round ligament that supports the womb. In either case, part of the intestine or fatty tissue protrudes into the groin at the top of the inner thigh. Inguinal hernias occur because of weakened muscles usually associated with aging and repeated strains (through

physical exertion, obesity, pregnancy, or frequent coughing, for example) on the abdominal and groin areas. In an umbilical hernia, fatty tissue or part of the intestine pushes through the abdomen near the naval. Adults may experience this by straining the abdominal area, being overweight, having a long-lasting heavy cough or after giving birth. In an incisional hernia, tissue protrudes through the site of an abdominal scar from a pelvic operation. In slightly different scenarios, a hiatal hernia involves part of the stomach pushing up into the chest cavity through an opening in the diaphragm, and an epigastric hernia is a protrusion between the naval and the sternum. Although some hernias resolve spontaneously, many do not; some form of hernia surgery is then necessary. These options are discussed in a later chapter.

2.2.2.11.5 Female breast

In almost every database in which 'spirituality' can be linked with 'female breast' the outcome is almost wholly concerned with spiritual aspects of breast cancer and its management. I will deal with these under the section on reconstruction after cancer in general. In relation to the arts, there is a significant literature and gallery devoted to the breast; it does not seem appropriate to have a discourse about the beauty of these tissues here, especially as these factors do not really relate to the structure and function. Again, I will cover some of these aspects when discussing aesthetics of reconstructed breasts later.

Female breasts contain fatty and non-fatty tissues. The non-fatty tissues, collectively referred to as fibroglandular tissue includes the breast lobes and breast ducts and the fibrous connective tissue, while fatty tissue fills in the spaces between glandular and fibrous tissue and largely determines breast size. Embedded in the breast's fatty and fibrous tissue are 15 to 20 glands called lobes, each of which has many smaller lobules that produce milk. Ducts carry milk to the nipple, which is located in the middle of the areola, which is the darker area surrounding the nipple. Anatomically, the adult breast sits above the pectoralis muscle, which is itself over the ribcage. The breast tissue extends horizontally from the edge of the sternum out to the midaxillary line. The breast tissue is encircled by a thin layer of fascia. The blood supply comes primarily from the internal mammary artery, which runs underneath the main breast tissue. The lymphatic vessels of the breast flow in the opposite direction of the blood supply and drain into lymph nodes. Most lymphatic vessels flow to the axillary lymph nodes, while a smaller number flow to internal mammary lymph nodes located deep in the breast.

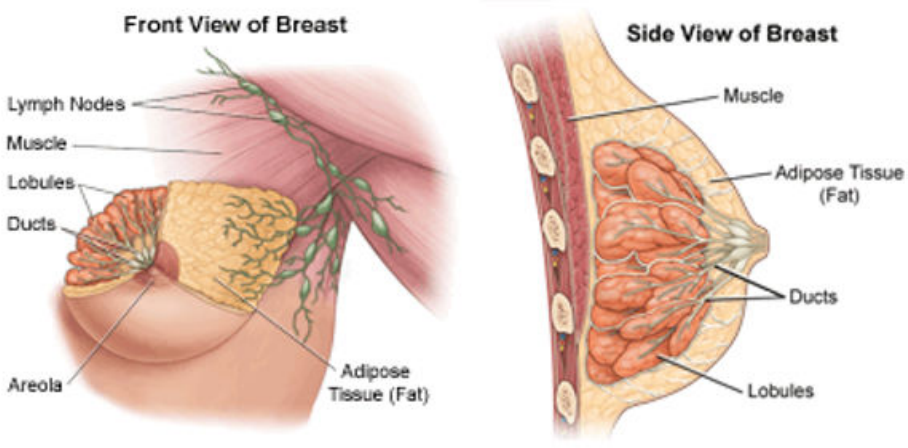

Figure 2.44. Components of the female breast.

The male breast structure is nearly identical to the female breast, except that the male breast tissue lacks the specialized lobules, since there is no physiologic need for milk production by males.

2.3 MOLECULAR AND MICROSTRUCTURAL CHARACTERISTICS

It is now time to move on from macrostructures and functions to the smaller, molecular, and cellular levels. This section deals with molecules and the following section with cells. I should emphasize two points at the beginning. First, it is almost impossible to provide, and follow, a simple precise classification of the nomenclature with respect to these molecules. This largely arises because of the multitude of molecules that are hybrid, as with glycoproteins and proteoglycans, so that they could fit into one, or other, or both categories represented in their names. In addition, classifications based on molecular structure do not necessarily categorize functions very well. Thus, in biochemical terms there are usually four defined types of large molecules, proteins, carbohydrates, lipids, and nucleic acids. In the section that follows, I include the first three, but have preferred to separate out nucleic acids into a different section, along with genes and chromosomes.

Secondly, it might be considered inappropriate to discuss molecular scale entities in a discourse on reconstructive technologies since we have historically reconstructed our bodies at the macroscale anatomical level, and mostly still do so today. However, as will already have become evident, reconstruction can be based on cellular therapies and gene editing, and it would be totally remiss to exclude these from the discussion.

2.3.1 Large Molecules

2.3.1.1 Proteins

There are several ways in which the role of proteins in the human body can be presented. I prefer to start with the various functions of proteins so that it is easier to understand how the chemical and morphological structures have evolved in order to provide these functions.

2.3.1.1.1 Functions of proteins in the human body

I list the major functions and types of protein here roughly in order to their relevance to reconstructive technologies, although I admit that this is rather subjective.

- Structural proteins provide support to the body, for example, the proteins of connective tissues, such as collagen and elastin.
- Contractile proteins, such as actin and myosin, are involved in muscle contraction and movement.
- Hormones are proteins that co-ordinate bodily functions, for example, insulin controls our blood sugar concentration.
- DNA-associated proteins, for example, histones, regulate chromosome structure during cell division or the regulation of gene expression,
- Enzymes facilitate biochemical reactions.
- Antibodies are proteins generated by the immune system, including IgG antibodies that target foreign body proteins or antigens.

- Transport proteins move molecules around, for example, haemoglobin transports oxygen through the blood.

Details of some of these functions, and their relation to specific structures are given later in this section.

2.3.1.1.2 Amino acids and protein structures

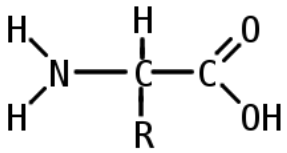

Figure 2.45a. The general structure of an amino acid molecule. The amino group is on the left, and the carboxylic acid group is on the right. The R group is unique to each amino acid.

Protein molecules consist of long chains of amino acids. An amino acid is an organic compound that contains both an amino ($-NH_2$) and carboxylic acid (-COOH) functional group. There are two major types, the alpha amino acids and beta amino acids, and one minor example, the gamma amino acids. The alpha amino acids have carboxylic acid and amine groups on the adjacent carbon atoms, whereas in beta amino acids the amine group is attached to the secondary carbon atom from the carboxylic acid group. The chains of amino acids assemble to form proteins *via* amide bonds, known as peptide linkages. The different side-chain group (the R-group in the above Figure 2.45a) determines the unique properties of each amino acid, the characteristics of different proteins then being determined by which amino acids it contains, how these are arranged in the chain, and the complex interactions that the chain makes with itself and the environment.

There are more than 100,000 unique proteins in the human body, with around 20,000 unique protein encoding genes being responsible for them. Although there are hundreds of amino acids found in nature, only about 20 amino acids, all alpha amino acids, are needed to make all the proteins found in the human body.

The principal amino acids that comprise these proteins are alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val).

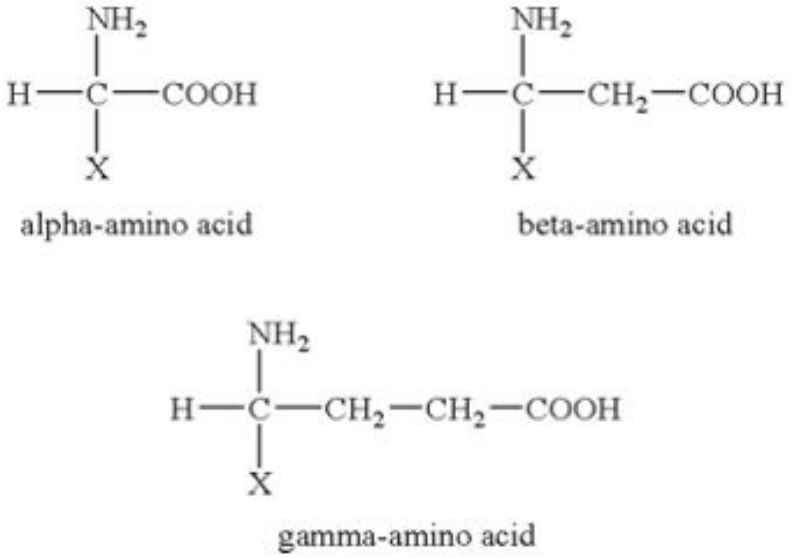

Figure 2.45 b. Structures of alpha, beta and gamma amino acids. The alpha form is the most common and the gamma form, the least.

The amino acids listed above are characterized as essential, non-essential or conditional. This is not related to their ultimate function but to their synthesis. Those which cannot be synthesized or produced by the body and must be obtained food from sources are the essential amino acids. On the other hand, those which are synthesized within the body and are not dependent on food sources are the nonessential amino acids; deficiencies related to these reduce the ability to make proteins that are required for the repair, growth, and maintenance of cells. Some of the amino acids which are usually not essential may become essential in times of stress, for example with prematurity in infants, and are referred to as conditional amino acids.

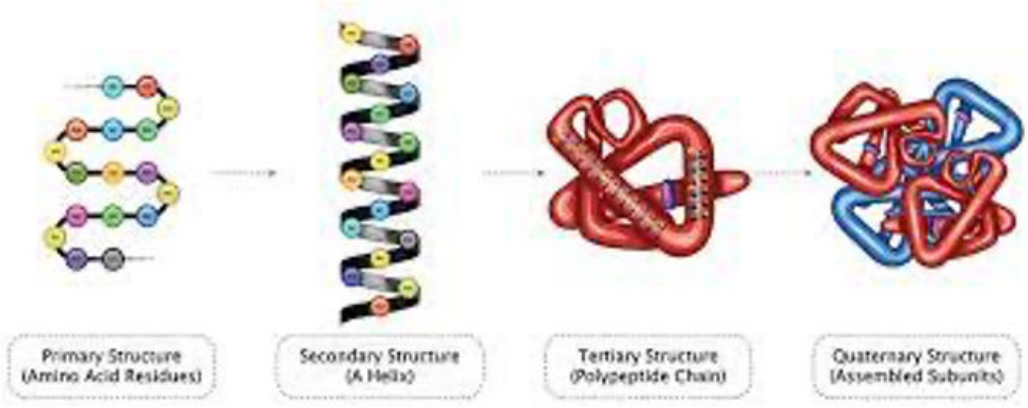

Figure 2.45 c. Primary, secondary, tertiary and quaternary structures of proteins

Although there are only these 20 amino acids commonly seen in the body, they can be arranged in a vast number of ways when creating the polymeric proteins, hence providing the variety of three-dimensional structures. The simple sequencing of the amino acids in a protein is known as its primary structure. The secondary protein structure depends on the local interactions between parts of a protein chain, affect folding and the three-dimensional shape of the protein. There are two main variations here. First there is the α-helix wherein N-H groups in the backbone form a hydrogen bond with the C=O group of the amino acid 4 residues earlier in the helix. Secondly there are β-pleated sheets where N-H groups in the backbone of one strand form hydrogen bonds with C=O groups in the backbone of a fully extended strand next to it. The tertiary structure of proteins refers to the overall 3D shape, influenced by the presence polar, nonpolar, acidic, and basic R groups on the protein. The quaternary protein structure relates to the orientation and arrangement of subunits in proteins with multi-subunits. Proteins fold up into specific shapes according to the sequence of amino acids in the polymer, and the protein function is directly related to the resulting 3D structure. Proteins may also interact with each other or other macromolecules to create complex assemblies in which they can develop functions that were not possible in a standalone protein.

2.3.1.1.3 Protein Synthesis

As will be seen in a later section, a gene is a segment of a DNA molecule that contains the instructions needed to make a unique protein. Each cell uses a different combination of genes to build the particular proteins it is designed to produce. Protein synthesis in the cells has two main stages. The first is transcription, which takes place in the cell nucleus. From two parallel strands of DNA, one acts as a template to produce the messenger molecule, mRNA. The enzyme RNA polymerase binds itself to a particular site (promoter region) in one of the DNA strands that will act as a template. The mRNA strand continues to elongate until the polymerase reaches a 'terminator region' in the template. Translation takes place in the cell cytoplasm and is initiated as soon as the transcribed mRNA enters the cytoplasm. The ribosomes immediately attach to the mRNA at a specific site, called the start codon. An amino acyl tRNA also binds at the mRNA strand. As the ribosomes move along the mRNA strand, the amino acyl tRNA brings amino acid molecules, one by one, in a stage called elongation. At the termination phase, the ribosomes read the last codon of the mRNA strand, and the polypeptide chain is released.

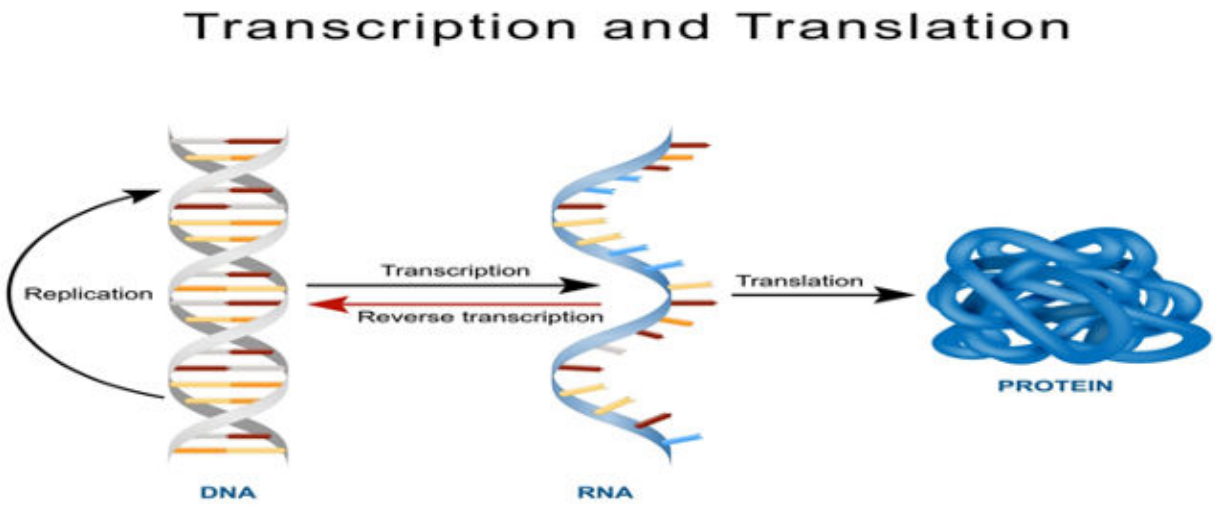

Figure 2.46. Protein synthesis.

2.3.1.1.4 Protein turnover and homeostasis: folding, unfolding and degradation

Human proteins have variable stability; they are regularly degraded but there is a large variation in their lifetimes. The balance between protein synthesis and degradation controls protein abundance, and dysregulation of these processes plays a substantial role in the pathogenesis of many diseases and in the overall ageing process[128]. One of the most important fundamental aspects here is the presence of molecular chaperones within cells that regulate protein synthesis, folding, folding and turnover[129]. The normal functionality of cells and organisms crucially depends on the ability of proteins to fold correctly into their native form, at all levels of the structures discussed above. Because of the complexity of the folding process, and the sheer number of reactions taking place all the time, it would be very surprising if misfoldings never took place. To compensate for this, several intracellular regulatory systems have evolved that sense and respond to misfolded proteins. The main mechanisms involve these chaperones, which are diverse families of multidomain proteins, much like enzymes but which operate on a much wider range of substrates. They are, effectively, the quality control system of protein synthesis, ensuring that proteins are correctly folded and functional at the right place and time. Under disease or other stress conditions, misfolded proteins can be unfolded, disaggregated and then re-folded, or targeted for disposal by proteolytic enzymes.

The endoplasmic reticulum (which is described in a later section) is a major site for this quality control procedure. However, alterations in the function of the ER can result in the accumulation of unfolded or misfolded proteins, a cellular condition referred to as ER stress; this engages the unfolded protein response, an adaptive reaction that reduces unfolded protein load to maintain cell viability and function. In conditions of chronic or irreversible ER stress, cell death by apoptosis may be triggered. Chronic ER stress has been linked to the occurrence of many diseases, including cancer and neurodegeneration.

Protein homeostasis is a significant factor in the ageing process[130]. Protein turnover is an effective way of maintaining a functional proteome in healthy individuals, as older, and potentially damaged, proteins are destroyed and replaced by newly synthesized copies. Proteins may be damaged under several conditions. Oxidative damage can have a devastating effect on the structure and activity of proteins and may even lead to cell death. The sulfur-containing amino acids cysteine and methionine are particularly susceptible to damage by reactive oxygen and chlorine species. Protein aggregation is the formation of non-native high molecular weight structures, which incapacitates the proteins; this is particularly seen in neurodegenerative diseases such as Huntington's and Alzheimer's disease. Deamidation is the non-enzymatic loss of amide groups on the side chains of asparagine and glutamine residues, which affects the charge on the protein and may cause cross-linking. The control of protein degradation is, obviously, a key element of turnover[131]; protein lifetimes vary, from minutes to days, so that the degradation machinery has to be both expansive but also selective. There are two pathways that control the intracellular protein degradation, the Ubiquitin-Proteasome System and the Autophagy Lysosome System. Anomalies in these systems may be associated with impaired development, cancer, and a range of disorders, collectively referred to as proteinopathies, which involve aggregation of protein entities[132].

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[128] Toyama BH and Hetzer MW, Protein homeostasis: live long, won't prosper, *Nature Reviews in Molecular Cell Biology*, 2013;14(1):55-61. doi:10.1038/nrm3496.

[129] Saibil H, Chaperone machines for protein folding, unfolding and disaggregation, *Nature Reviews in Molecular Cell Biology*, 2013;14(10):630-42. doi:10.1038/nrm3658.

[130] Krisko A and Radman M, Protein damage, ageing and age-related diseases, *Open Biology*, 2019;9:180249, doi:10.1098/rsob.180249.

[131] Marrero MC and Barrio-Hernandez I, Towards understanding the biochemical determinants of protein degradation rates, *ACS Omega*, 2021;6:5091-100. Doi:10.1021/acsomega.Oc05318.

[132] Ciccocioppo F, Bologna G, Ercolino E, *et al,* Neurodegenerative diseases as proteinopathies-driven immune disorders, *Neural Regeneration Research*, 2020;15(5):850-6, doi:10.4103/1673-5374.268971.

2.3.1.1.5 Recombinant proteins and protein engineering

The discussion so far has only concerned proteins occurring naturally in the human body. In recent years, it has become possible to manipulate such proteins such that the modified protein has properties that can be used for industrial or medical applications[133], including some that are relevant to reconstructive procedures.

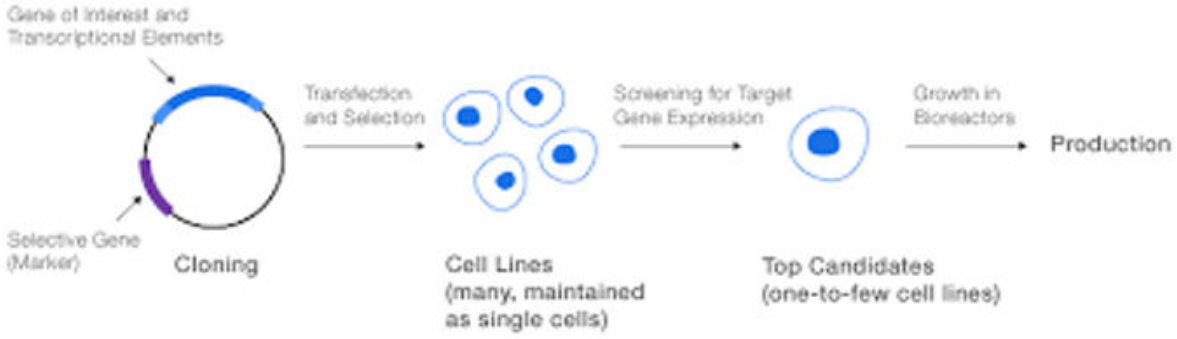

Figure 2.47. Production of recombinant proteins; the required gene, together with agents necessary for transcription, is cloned into a suitable expression vector with a selective gene. The final vector is transfected into host cells, which are screened for production of the desired protein and transferred to a bioreactor for large-scale production.

One of the most prominent manipulation techniques here involves recombinant proteins. These are proteins encoded by recombinant DNA that has been cloned in an expression vector that supports expression of the gene and translation of messenger RNA. Modification of the gene by recombinant DNA technology can lead to expression of a mutant protein. Recombinant protein is a manipulated form of native protein, which is generated in various ways in order to increase production of proteins, modify gene sequences, and manufacture useful commercial products. Recombinant protein production begins at the genetic level, where coding sequence for the protein of interest is first isolated and cloned into an expression plasmid vector. Most recombinant proteins for therapeutic use are from humans but are expressed in microorganisms such as bacteria, yeast, or animal cells in culture. Many recombinant proteins require protein modifications, such as glycosylation, that are available only in eukaryotic cells.

Recombinant proteins are commonly used to produce pharmaceutical products, protein-based polymers for drug delivery, antibodies and enzymes for disease treatment, protein scaffolds for tissue engineering, as well as for a myriad of other uses. The first recombinant protein used in treatment was recombinant human insulin in 1982. The recombinant protein industry has rapidly grown. Today, clinically used recombinant proteins include some hormones, interferons, growth factors, blood clotting factors and a variety of crucial enzymes.

[133] Puetz J and Wurm FM, Recombinant proteins for industrial versus pharmaceutical purposes: A review of processes and pricing, *Processes,* 2019;7:476, doi:10.3390/pr7080476.

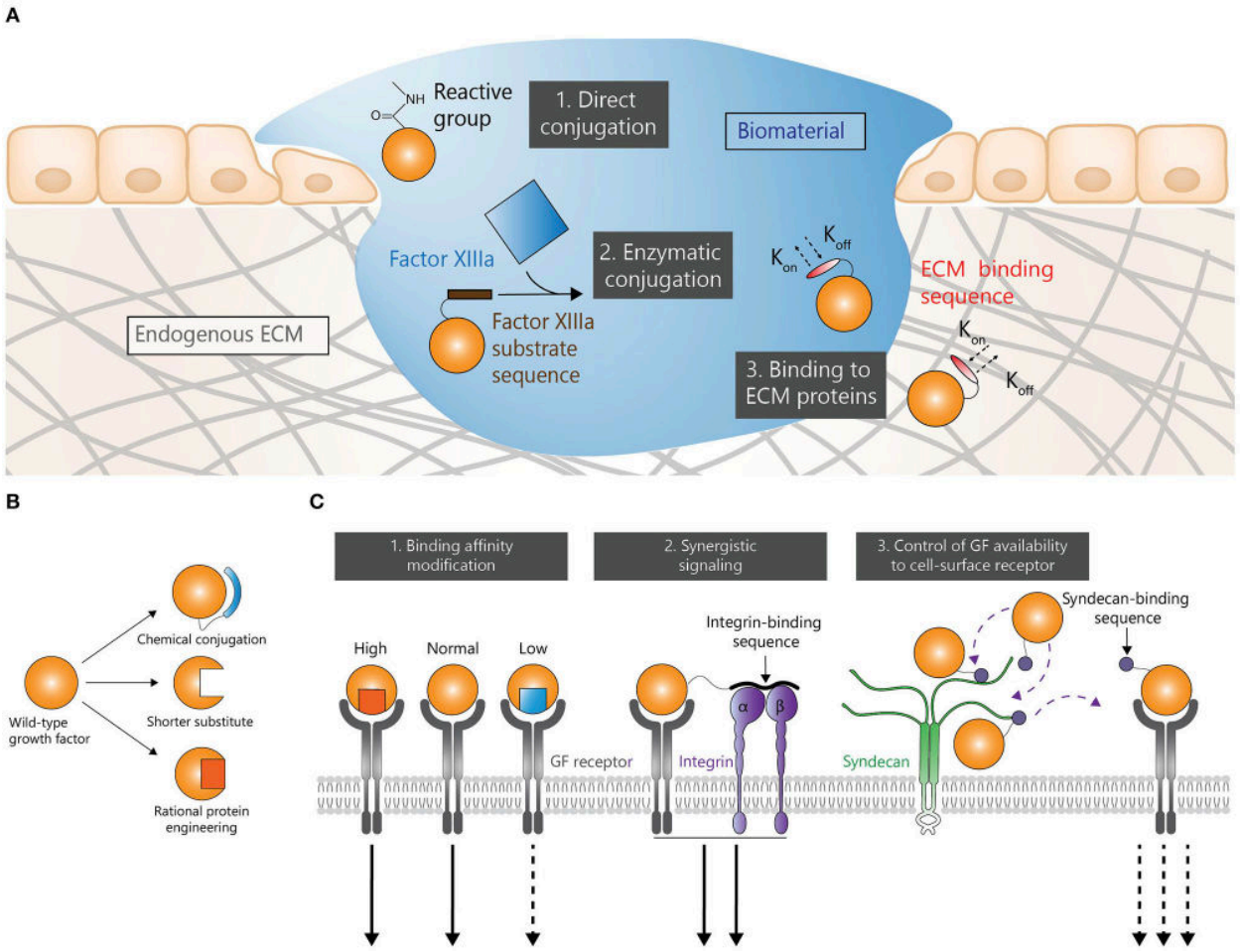

Figure 2.48. Growth factor engineering strategies aimed at (A) controlling spatial presentations by incorporation in biomaterials, (B) improving stability and (C) improving signaling [158].

On a broader scale, manipulating proteins within the general scheme of protein engineering has become a major contribution to biotechnology in recent years, although with most emphasis on product biosynthesis[134]; examples include the improvement of enzymatic activities and strengthening enzyme stability. One aspect that has considerable potential for regenerative medicine technologies is that of growth factor engineering[135]. Growth factors, defined as any of a group of proteins that stimulate the growth of specific tissues, especially playing a role in cellular differentiation and cell division. It follows that they are critical for processes within the phenomena of tissue repair and regeneration. It has been an ambition for some time to use growth factor products therapeutically to enhance these processes, but there are profound limitations associated with their short half-life, rapid diffusion from the site of delivery and high cost. The use of supraphysiological doses is not possible because of serious side effects[136]. Much attention has been given to growth factor engineering by immobilization within biomaterials (A in the Figure abve) and introducing mutations at know cleavage sites to improve protease resistance (B).

[134] Li C, Zhang R, Wang J, *et al,* Protein engineering for improving and diversifying natural product biosynthesis, *Trends in Biotechnology*, 2020;38(7):729-44, doi:10.1016/tibtech.2019.12.008.
[135] Ren X, Zhao M, Lash B, *et al,* Growth factor engineering strategies for regenerative medicine applications, *Frontiers in Bioengineering and Biotechnology,* 2020;7:469. doi:10.3389/fbioe.2019.00469.
[136] Baldo BA, Side effects of cytokines approved for therapy, *Drug Safety*, 2014;37:921-43. doi:10.1007/s40264-014-0226-z.

2.3.1.1.6 Some specific proteins relevant to reconstructing the body

Many different proteins have some relevance to reconstructive technologies. A few of these are described in this section.

Collagen[137]

Collagen proteins are composed of a triple helix, which generally consists of two identical α1 chains and an additional α2 chain that differs slightly in its chemical composition. As noted below, there are many different forms of collagen, the most common motifs in the amino acid sequence of being are Gly-Pro -X and Gly-X-Hyp, where X is any amino acid other than Gly, Pro or Hyp. Note that Hyp, hydroxyproline, is a product of posttranslational hydroxylation of proline and is not a typical amino acid.

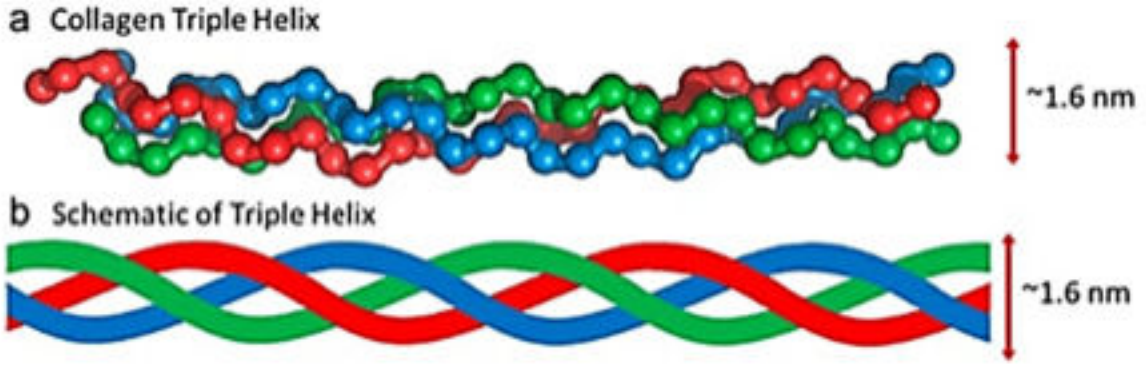

Figure 2.49. The triple helix structure of collagen proteins.

Individual collagen triple helices, known as tropocollagen, assemble in a hierarchical manner that ultimately leads to the macroscopic fibers and networks observed in many tissues. The categories of collagen include the classical fibrillar and network-forming collagens, the fibril-associated collagens with interrupted triple helices, membrane associated collagens with interrupted triple helices, and multiple triple helix domains and interruptions.

There are 28 distinct types of collagen, although only a few are widely distributed in the human body. Up to 90 percent of the body's collagen is type I collagen, which is found in the skin, bones, blood vessel walls, fibrous cartilage and in scar tissue. Type II collagen is the basis for articular cartilage and hyaline cartilage, making up 50% of all protein in cartilage and 85–90% of collagen of articular cartilage. Type III collagen provides the structure of muscles, as well as organs and blood vessels. Comprised of meshy reticular fibers, this form is the second most prevalent after type I.

The hierarchical structure of collagen provides a range of mechanical properties, especially as the fibrils are anisotropic. The tensile strength of collagen in tendon is in the region of 100 MPa. The Young's modulus of the tropocollagen monomer is 7 GPa and that of rat tail collagen is 11 GPa, respectively; the shear modulus of dehydrated fibrils of type I bovine tendon collagen 33 MPa. Hydration of these fibrils reduces the shear modulus significantly, whereas cross-linking increases it. Excessive cross-linking results in very brittle collagen, a common symptom of aging.

[137] Shoulders MD and Raines RT, Collagen structure and stability, *Annual Reviews of Biochemistry*, 2009;78:929-58. doi:10.1146/annurev.biochem.77.032207.120833.

Bovine collagen is useful for some biomedical purposes, but it suffers from potential immunogenicity, heterogeneity, and loss of structural integrity during the isolation process. The heterologous production of collagen is not easy because of the difficulty of incorporating post-translational modifications and the need to use complex expression systems. Much attention has therefore been paid to chemical synthesis of collagen for medical applications.

There are several disorders that affect collagen[138], most being either of genetic or autoimmune origin. In the former category are osteogenesis imperfecta, epidermolysis bullosa and Ehlers-Danlos syndrome. Osteogenesis imperfecta is a group of heritable disorders that arise from impairment of collagen maturation. All tissues rich in type I collagen may be affected, giving rise to clinical signs of abnormal bone formation and fragility, joint laxity and hypermobility and dentinogenesis imperfecta. Epidermolysis bullosa is a group of rare genetic disorders that are characterized by the formation of vesicles and bullae on the skin and mucous membranes. Ehlers-Danlos syndrome, otherwise known as tenascin-X deficiency syndrome is characterized by hyperextensibility of the skin and hypermobility of joints. The two most prominent autoimmune collagen disorders are systemic lupus erythematosus and systemic sclerosis. The former is a multifactorial collagen or connective tissue disease, with common symptoms of fever, weight loss, fatigue and malaise, often with oral and cardiac manifestations. Systemic sclerosis is a progressive disorder, with extensive fibrosis of many tissues, associated with hyperplastic changes in collagen.

Elastin[139]

Elastin is the protein that is primarily responsible for the extensibility and elastic recoil of many tissues, including large arteries, heart valves, pulmonary tissues, skin, and some ligaments and cartilages. Elastic fibers are composed of an insoluble polymerized elastin core with peripheral microfibers; their networks are essential for maintenance of normal physiological functions in many tissues and organs. Owing to its extensive crosslinked structures, elastin is a long-lived protein that degrades slowly in healthy tissues, with a half-life of about 70 years. Its polymeric molecule has hydrophobic properties; in the presence of water, it exists with a rubbery character, with a low elastic modulus.

Over 75% of the sequence of elastin consists of just four non-polar amino acids, glycine, valine, alanine, and proline. Tropoelastin, the soluble precursor of elastin with a molecular weight of about 60 kDa, is only encoded by a single gene, *ELN*, which is located on chromosome 7q11.1-21.1 with a size of 45 kb. Tropoelastin has two major domains, alternating hydrophobic regions and hydrophilic cross-link domains. The hydrophobic domains are rich in non-polar amino acids, particularly glycine, valine, proline, and alanine, which often appear in repeats of 3–6 peptides, the composition of which facilitates the hydrophobic interactions that results in the elastic properties. The hydrophilic cross-link domains are rich in alanine and lysine and are necessary to form insoluble elastin. Tropoelastin is the most elastic and expansive monomer protein, extending up to eight times its length. The molecular shape is asymmetric, with a coil-like structure in the N-terminal region and hinges which act as a bridge to connect the N-terminal region to the C-terminal region. Tissues rich in elastin include the aorta and major blood vessels (28–32% dry mass), the lungs (3–7%), elastic ligaments (50%), tendons (4%), and the skin (2–3%).

[138] Myllyharju J and Kivirikko KI, Collagens and collagen-related diseases, *Annals of Medicine,* 2001;33(1): 7-21, doi: 10.3109/07853890109002055.

[139] Wang K, Meng X and Cuo Z, Elastin structure, synthesis, regulatory mechanism and relationship with cardiovascular diseases, *Frontiers in Cell Developmental Biology,* 30 November 2021, doi:10.3389/fcell.2021.596702.

Keratin[140]
The term 'keratin' denotes intermediate filament-forming proteins with specific physicochemical properties and produced by any vertebrate epithelia. They are found in many species across a range of birds, amphibians, and mammals, and are the major structural proteins of hair, nails, scales, feathers, horns, hooves, claws and the outer layer of mammalian skin. In humans, there are over 50 functional keratin genes, being expressed in highly specific patterns related to the epithelial type. Keratins provide mechanical stability to the epithelial cytoskeleton, but some also have regulatory functions and are involved in intracellular signaling pathways. Keratins are resistant to digestion by most proteases and are insoluble in dilute acids, alkalines, water and organic solvents.

Keratin proteins are either alpha-keratins or beta-keratins, based on the geometry of their polypeptide chains. Alpha-keratins, which are found in the hair, the skin, and the wool of mammals, are primarily fibrous and helical in structure. Beta-keratins, which occur in birds and reptiles, consist of parallel sheets of polypeptide chains. The amino acid composition also varies, depending on the tissue in which it occurs and its function. Cysteine residues, are covalently linked via disulfide bonds, forming cystines, which are responsible for the great stability of keratin.

Actin[141] and **Myosin**[142]
Actin and myosin are two groups of protein that act together in functions related to mobility, especially muscle contraction. Actin is the most abundant type of protein in most eukaryotic cells; actin proteins are highly conserved and engage in many protein-protein interactions. A very important feature is their ability to transition between two states, the monomeric (G-actin) and filamentous (F-actin) states, this transition occurring under the control of nucleotide hydrolysis and a large number of actin-binding proteins. Essentially, F-actin filaments are linear polymers of globulin actin. In humans there are three main actin isoforms, including three α-isoforms of skeletal, cardiac, and smooth muscles, and the β- and γ-isoforms which are expressed in both non-muscle and muscle cells. Actin filaments can create linear bundles, two-dimensional networks, and three-dimensional gels. They occur as microfilaments in the cytoskeleton and as thin filaments, as part of the contractile apparatus, in muscle and non-muscle cells. The actin filaments in muscles are separated by actin-binding proteins, α-actinin that binds two actin filaments, while leaving space for myosin. In many cells, they underlie the plasma membrane and may be assembled at the cell periphery from adhesion sites or sites of membrane extension. Actin proteins play essential roles in cell division, cell motility, and cell signaling.

The myosin superfamily of proteins consists of more than 30 individual structures in humans. They contain a highly conserved globular head, which has ATPase and actin-binding sites, and a rod-like tail which controls protein-protein interactions. They occur in almost all eukaryotic cells and take part in many functions, including endocytosis, cytokinesis, organelle transport and cytoskeletal support. They exist in two groups, the conventional myosins, which are filaments of two heavy chains and two pairs of light chains, and the non-filamentous unconventional myosins. The former are found in both muscle and non-muscle cells, acting as the molecular motors that drive sarcomeric contractions through the conversion of chemical energy, in the form of ATP, into mechanical energy.

Actin filaments, together with myosin, are responsible for most types of cell movement, the best example being seen with skeletal muscle contraction. The contractile elements of the cytoskeleton exist in highly

[140] Bragulla HH and Homberger DG, Structure an functions of keratin proteins in simple, stratified, keratinized and cornified epithelia, *Journal of Anatomy,* 2009;214:516059, doi:10.1111/1469-7580.2009.01066.x
[141] Dominguez R and Holmes KC, Actin structure and function, *Annual Reviews in Biophysics*, 2011;40:169-86, doi:10.1146/annurev-biophys-042910-155359.
[142] Tardiff JC, Myosin at the heart of the problem, *New England Journal of Medicin*e, 2004;3515424-6. doi:10.1056/NEJMp048142.

ordered arrays, which are responsible for the characteristic pattern of cross-striations. Skeletal muscles are essentially bundles of fibers, which are single large cells, the cytoplasm of which consists of myofibrils, in which are thick filaments of myosin and thin filaments of actin, of diameter 15nm and 7nm respectively. Each myofibril consists of a chain of contractile units, the sarcomeres, of length just over 2μm. The ends of each sarcomere are distinguished by the Z disc. During muscle contraction, each sarcomere shortens, bringing the Z disks closer together as actin and myosin filaments slide past each other.

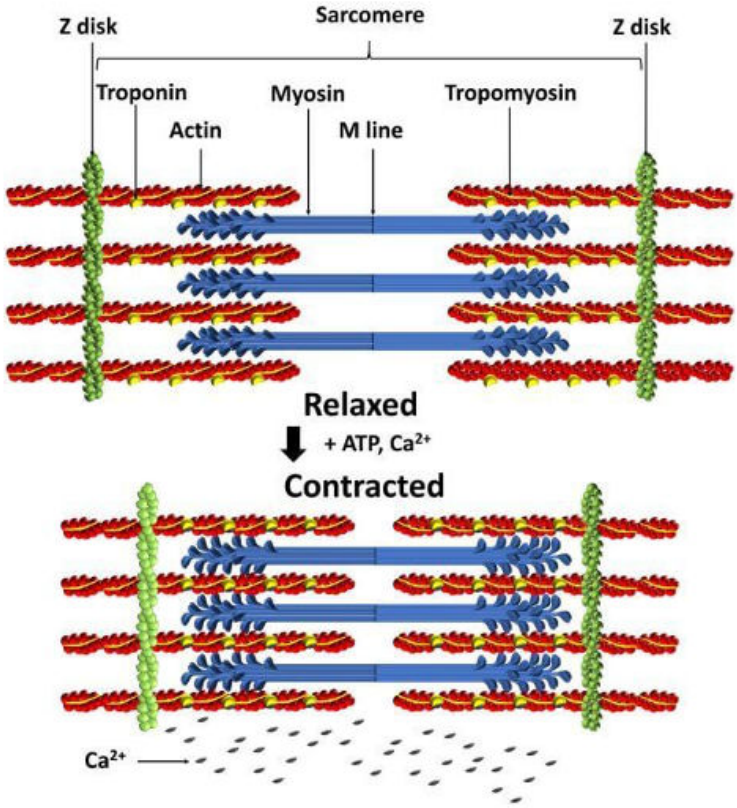

Figure 2.50 Muscle contraction and the sliding of actin and myosin filaments.

Immunoglobulins[143]

Immunoglobulins are heterodimeric proteins (macromolecules formed by two different proteins) that are composed of two heavy and two light chains. Functionally, they have variable domains that bind antigens and constant domains that determine specific effector functions, such as binding to Fc receptors. There are 5 main classes of heavy chain constant domains. Each class defines the IgM, IgG, IgA, IgD, and IgE isotypes. IgG can be split into 4 subclasses, IgG1, IgG2, IgG3, and IgG4, each with its own properties, and IgA can be either IgA1 or IgA2.

More specifically, immunoglobin molecules (widely referred to as antibodies) consist of two identical heavy chains and two identical light chains**,** which consequently give the antibody two antigen-

[143] Schroeder HW and Cavacini L, Structure and function of immunoglobulins, *Journal of Allergy and Clinical Immunology*, 2010;125:S41-52. doi:10.1016/j.jaci.2009.09.046.

binding sites**.** Disulfide bonds bind the heavy chains to each other and to the light chains. The heavy and light chains also consist of several amino-acid sequences, each corresponding to a protein domain, which are the functional units of the antibody and correspond to a discrete, folded region of protein structure. Each light chain has two domains, one variable and one constant, and each heavy chain has one variable and three constant domains.

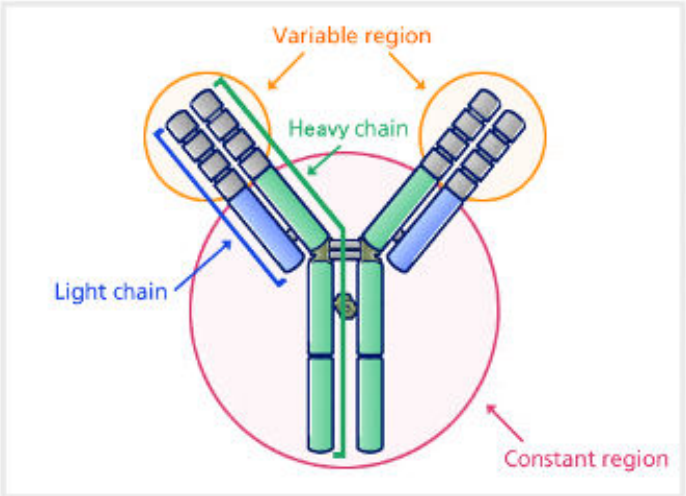

Figure 2.51. General structure of immunoglobulin molecule.

Each immunoglobulin molecule contains two variable regions and one constant region**.** The Fab regions (fragment antigen binding) contain the variable domains of the light and heavy chains, which give the antigen specificity. Each Fab region also contains two constant domains**,** one each from the heavy chain and light chain components. The Fc regio**n** (fragment crystallizable) consists of the remaining constant domains from the two heavy chains. The Fc region interacts with different immune cells and mediates various functions. The constant region incorporates the constant domains from both the Fab and Fc parts. The heavy chain constant domains define antibody class and are the same for all antibodies of the same class.

Antibodies are classified according to heavy chain type, which is encoded by a gene on chromosome 14. The different classes are IgG, IgA, IgM, IgD and IgE, in descending order of abundance in serum.

IgG is the most abundant immunoglobulin, being present on the surface of mature B-cells and in serum. IgG is the only immunoglobulin that can cross the placenta, thereby providing passive immunity to the fetus, infants then having high IgG levels in the first 3-6 months. IgA is the most prevalent antibody in secretions such as saliva and mucous. IgA antibodies are resistant to enzymatic digestion and act principally as neutralizing antibodies. Breast milk and colostrum have high levels of IgA, protecting against infections in breast-fed babies. IgA neutralizes pathogens at mucosal surfaces and hinders their attachment to epithelial receptors by binding to their ligands on pathogens or toxins. IgM antibodies are expressed on the surface of B-cells as monomers but secreted as pentameters, which have five antibodies connected by a joining chain. IgM has high avidity, meaning the antibody-antigen complex is strong, but low affinity, so the strength of a single epitope-antibody interaction is weak. IgD has a role in B-cell and antibody production. IgE is mainly found on mast cells but is also present in blood and extracellular fluid. It is associated with allergy, particularly type I hypersensitivity reactions, including atopic disease and anaphylaxis. IgE is also part of the body's response to parasitic infections.

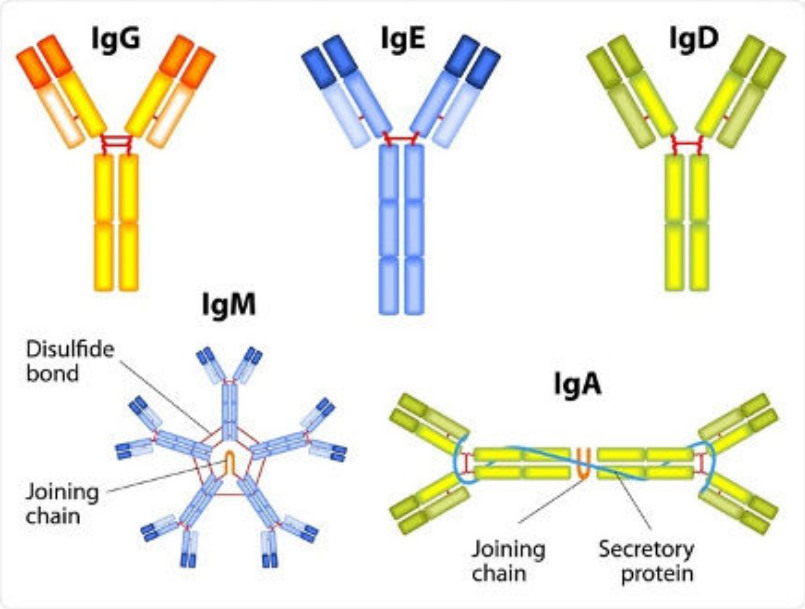

Figure 2.52. The five classes of immunoglobulins.

There are more than 200 different forms of primary immune deficiency diseases, most being quite rare. Among these are chronic granulomatous disease, which occurs when phagocytic cells are unable to kill certain bacteria and fungi, making people highly susceptible to some bacterial and fungal infections, common variable immunodeficiency, which is caused by certain genetic abnormalities that result in a defective capability to produce normal amounts of protective antibodies, and glycosylation disorders which can disrupt the immune system, resulting in immunodeficiency, causing extensive and severe symptoms.

Insulin[144]

Insulin is a small but fully functioning protein, possessing most of the typical protein structural features, but also playing a central role in the regulation of metabolism, especially carbohydrate metabolism. It is a globular protein containing two chains, the A-chain consisting of 21 residues and the B chain with 30 residues. The molecule is linked by three disulfide bridges, two inter-chain and one intra-chain. Several different monomeric, dimeric and hexameric crystal structures exist, and transitions between these forms is critical to insulin functions, and various mutations in these structures can cause conformational changes that are important in the induction of the disease diabetes mellitus. The first step in hormonal control of metabolic processes is the specific binding of the insulin to the insulin receptor, which triggers a series activation of tyrosine kinase, insulin then increasing sugar transport and protein synthesis, with activation of some enzymes and deactivation of others.

This subject is covered in more detail in the sections on diabetes and its treatment.

[144] Hua Q, Insulin: a small protein with a long journey, *Protein Cell,* 2010;1(6):537-51, doi:10.1007/s13238-010-0069-z.

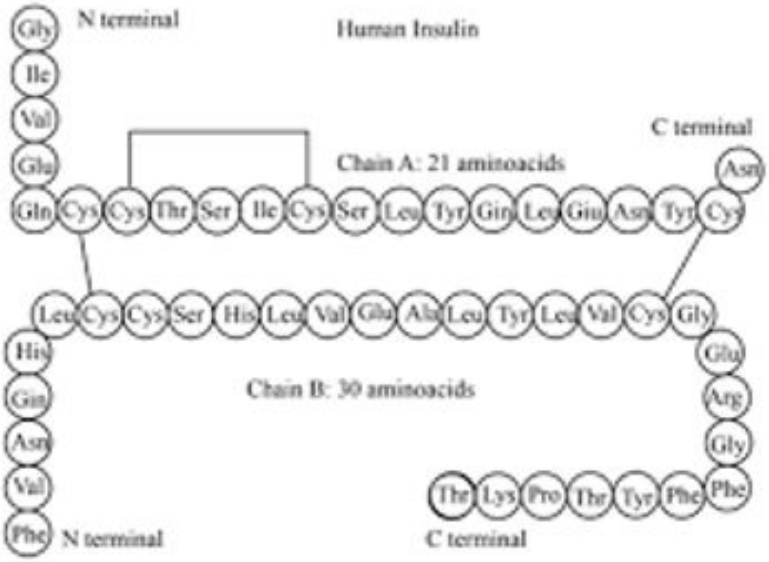

Figure 2.53. Structure of insulin.

Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor ß family (TGF ß). They are multifunctional cytokines, with both osteogenic and non-osteogenic activities. More than a dozen BMPs have been identified in vertebrates, although terminology is a little confusing since they were discovered and named in different phases of knowledge about growth and differentiation processes.

BMPs interact with specific receptors on the cell surface, referred to as bone morphogenetic protein receptors (BMPRs). Signal transduction through these receptors results in mobilization of members of the SMAD family of proteins, these pathways, involving BMPs, BMPRs and SMADs, being important in the development of the heart, central nervous system, and cartilage. They are also significant in embryonic development and early skeletal formation. BMP-2 acts as a disulfide -linked homodimer and induces bone and cartilage formation, playing a key role in osteoblast differentiation. BMP-3 also induces bone formation. BMP-4 regulates the formation of teeth, limbs and bone. It also plays a role in fracture repair, epidermis formation, dorsal-ventral axis formation, and ovarian follicle development. BMP-5 is involved in cartilage formation and BP-6 has a role in joint integrity in adults and controls iron homeostasis. BMP-6 plays a key role in osteoblast differentiation, induces the production of SMAD1 and is involved with renal development and repair.

It will be clear that BMPs are involved in far more tissues than bone, and BMP signaling pathways are responsible for cell growth, apoptosis and differentiation across the whole body, being involved in both tissue development and the pathogenesis of many diseases[145]. Within the skeletal system itself, BMPs regulate bone and cartilage development, also playing a role in adult bone homeostasis and fracture healing. Some BMPs, especially BMP-4, are crucial for maxillofacial development, and variants or mutations here are prominent risk factors for cleft lip and palate. Variability in gene expression of BMP-5 is a factor in osteoarthritis. In the cardiovascular and pulmonary systems, BMP-4 is a prominent signaling molecule. BMP-2 has a critical role in pulmonary development, and pulmonary arterial hypertension is

[145] Wang RN, Green J, Wang Z, et al, Bone morphogenetic protein (BMP) signaling in development and human disease*, Genes and Diseases*, 2014;1:87-105. doi:10.1016/j,gendis.2014.07.005.

often associated with germline mutations in BMP-2. BMP-7 is important in development of the eye, and germline mutations here cand prevent the formation of the lens.

Hemoglobin

Hemoglobin is the protein that is responsible for transportation of oxygen from the lungs to all parts of the body; it also imparts the red color to blood through its association with iron within that transport process.

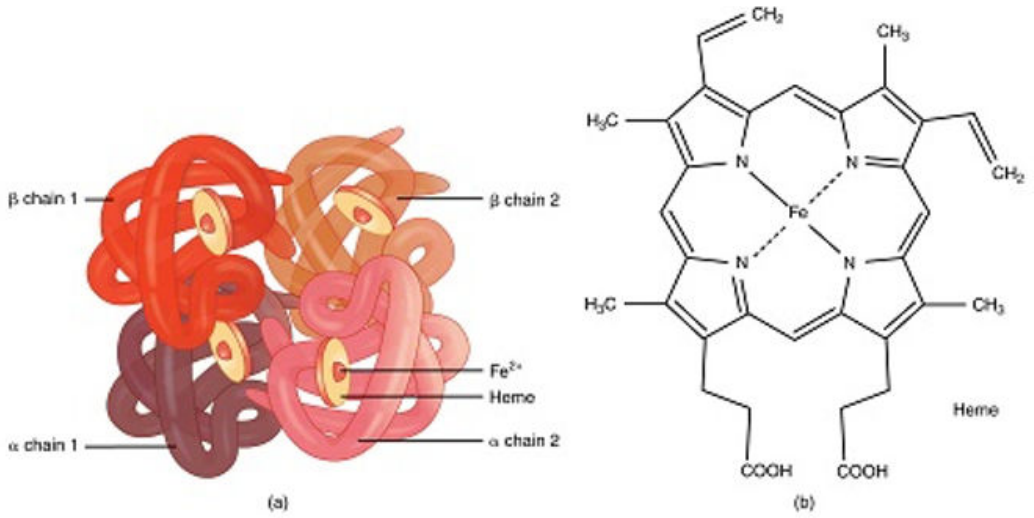

Figure 2.54. Structure of hemoglobin.

Hemoglobin has four sub-units, each with a polypeptide chain and a heme group. The latter has a ringlike organic compound, porphyrin, to which an iron atom is attached, *via* a link to the N atom of a histidine group. The polypeptide chains are of two types, the alpha and beta chains, which are similar in length but of differing amino acid sequences. Oxygen binds reversibly to the ferrous iron atom in each heme group, the extent of the binding varying with the partial pressure of oxygen. The oxygen-loaded form is known as oxyhemoglobin. Substances other than oxygen, such as carbon monoxide, are also able to combine with the ferrous atom; this binding is stronger than that achieved with oxygen, a principal factor in carbon monoxide poisoning. The fit between the polypeptide chains is crucial since it determines the access of oxygen to the iron. The adequacy of oxygen transport is dependent on this pathway and the affinity of hemoglobin for the oxygen. Any abnormalities along the pathway can lead to serious physiological problems. A major condition associated with hemoglobin abnormalities is sickle cell disease, where a single base-pair mutation in the gene for the beta-globin chain, leading to the formation of a distinct hemoglobin, which gives rise to the sickle-shape of red cells and subsequent change to their properties.

Enzymes

Enzymes are proteins that act upon substrate molecules, decreasing the activation energy necessary for a chemical reaction by stabilizing the transition state and speeding up reaction rates so they become physiologically relevant. They are usually highly specific and only bind certain substrates (at key locations known as active sites) for defined reactions. As with other proteins enzymes consist of amino acids within one or more polypeptide chains. This sequence of amino acids defines the primary structure, which in turn determines the 3D structure of the enzyme, including the shape of the active site. The active site is a groove or crevice on an enzyme in which a substrate binds to facilitate the catalyzed chemical reaction. Enzymes are specific since the conformation of amino acids in the active site stabilizes the

specific binding of the substrate. Some enzymes, called apoenzymes, are inactive until they are bound to a cofactor, which can be either metal ions or organic compounds that attach, either covalently or noncovalently, to the enzyme. The cofactor and apoenzyme complex is called a holoenzyme.

There are six main categories of enzymes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. It is impossible to catalogue these here, or even to describe any in detail, but a few examples are briefly mentioned.

- Oxidoreductase enzymes catalyze the transfer of electrons from one molecule (the reductant or electron donor) to another (the oxidant or electron acceptor). These enzymes usually utilize NADP+ or NAD+ as co-factors. Examples include those that act on the CH-OH group of donors (alcohol oxidoreductases), on peroxide as an acceptor (peroxidases) and on the CH-NH_2 group of donors (e.g., monoamine oxidase).

- Transferase enzymes catalyze the transfer of specific functional groups from donor molecules to acceptor molecules. They are involved in very many reactions in the cell, such as the activity of coenzyme (CoA) transferase, which transfers thiol esters, and the action of N-acetyltransferase, within the pathway that metabolizes tryptophan.

- Hydrolases use water to break chemical bonds, usually dividing a large molecule into smaller ones. For example, lipases contribute to the breakdown of fats and lipoproteins and other larger molecules into smaller molecules such as fatty acids that are used for synthesis and as a source of energy. Glycosidases cleave sugar molecules off carbohydrates, peptidases hydrolyze peptide bonds and nucleosidases hydrolyze the bonds of nucleotides.

- A lyase catalyzes the breaking of chemical bonds by actions other than hydrolysis or oxidation, frequently forming a new double bond or ring structure. Examples include those that cleave carbon–carbon bonds, such as decarboxylases and those that cleave carbon–oxygen bonds, such as dehydratases.

- Isomerases convert a molecule from one isomer to another, facilitating intramolecular rearrangements. There is only one substrate, and the product has the same molecular formula but differs in spatial arrangement. They have important functions in glycolysis and carbohydrate metabolism.

- Ligases catalyze the joining of two large molecules by forming new chemical bonds, often by hydrolysis. They have important roles in DNA function, for example joining two complementary fragments of nucleic acid and repair single stranded breaks that arise in double stranded DNA during replication.

2.3.1.2 Carbohydrates

2.3.1.2.1 General structure and classifications

Carbohydrates have the general stoichiometric formula $(CH_2O)n$.

There are five primary functions of carbohydrates in the human body: energy production, energy storage, building macromolecules, sparing protein and lipid metabolism. The discussion about these functions centers on the principal carbohydrate in mammals, glucose, the structure of which is given later.

The primary role of carbohydrates is to supply energy to all cells; indeed, many cells prefer glucose as a source of energy compared to other compounds and some, such as red blood cells, are only able to produce cellular energy from glucose. The brain only uses glucose to produce energy and function. Cells that require energy remove the glucose from the blood *via* transport proteins in their membranes. Cellular respiration is basically a controlled burning of glucose. A cell uses many chemical reactions in multiple enzymatic steps to control the release of energy and its capture. The first stage in the breakdown of glucose is glycolysis, which occurs in a series of ten enzymatic-reaction steps. The second stage occurs in the mitochondria, where one carbon and two oxygen atoms are removed, yielding more energy.

Excess glucose in the body is stored as glycogen, largely in the muscle and liver. The highly branched molecule of glycogen may contain in excess of fifty thousand single glucose units, allowing for the rapid dissemination of glucose when needed to produce cellular energy. Prolonged muscle use can deplete the glycogen energy reserve, characterized by fatigue, it taking longer to transform the energy in fatty acids and proteins than with glucose. Although most absorbed glucose is used to make energy, some glucose is converted to ribose and deoxyribose, which are essential building blocks of macromolecules, such as RNA, DNA, and ATP. Glucose is also utilized to make the NADPH, which is important for protection against oxidative stress.

Carbohydrates are classified into three subtypes: monosaccharides, disaccharides, and polysaccharides. Monosaccharides are simple sugars, where the number of carbon atoms is typically between three and seven. If the sugar has an aldehyde group ((R-CHO), it is an aldose, and if it has a ketone group (RC(=O)R′), it is known as a ketose. The most important monosaccharide in humans is glucose ($C_6H_{12}O_6$), discussed below. Galactose and fructose are isomers of glucose; galactose is found in milk sugars and fructose in fruit sugars. They have a different arrangement of functional groups around the asymmetric carbon atom. Monosaccharides can exist as a linear chain or as ring-shaped molecules; in aqueous solutions they are usually found in ring form. Glucose in a ring form can have two different arrangements of the hydroxyl group around the anomeric carbon. If the hydroxyl group is below carbon number 1 in the sugar, it is in the alpha (α) position, and if it is above the plane, it is in the beta (β) position. Disaccharides form when two monosaccharides undergo dehydration, or condensation, during which the hydroxyl group of one monosaccharide combines with the hydrogen of another, forming a covalent bond. The covalent bond formed between two monosaccharides is known as a glycosidic bond. Common disaccharides include lactose, maltose, and sucrose. A polysaccharide is a long chain of monosaccharides linked by glycosidic bonds. The chain, which may contain several different types of monosaccharide, may be branched or unbranched; the molecular weight may be greater than 100,000 Daltons. Starch, glycogen, cellulose, and chitin are examples of polysaccharides.

Carbohydrates are not obvious subjects for serious discussion in a treatise on reconstruction of the body, but some of them do have vital functions within many tissues and may be used as components of reconstructive technologies. They are most usually discussed in the context of nutrition being, along with proteins and fats, one of the main nutrients in the human diet. Few of the more relevant carbohydrates are described briefly in the following section.

Glucose

Glucose ($C_6H_{12}O_6$) is a hexose, a simple monosaccharide containing six carbon atoms. Five of the carbons plus an oxygen atom form the pyranose ring, where each carbon is linked to hydroxyl and hydrogen side groups with the exception of the fifth atom, which links to a 6th carbon atom outside the ring, forming a CH_2OH group. From a chemical perspective, there are two enantiomers (mirror-image isomers), D-glucose and L-glucose, but in living organisms only the D-isomer exists. The ring structure may form in two ways, yielding alpha glucose and beta glucose, differing in the orientation of the hydroxyl group linked to the first carbon in the ring.

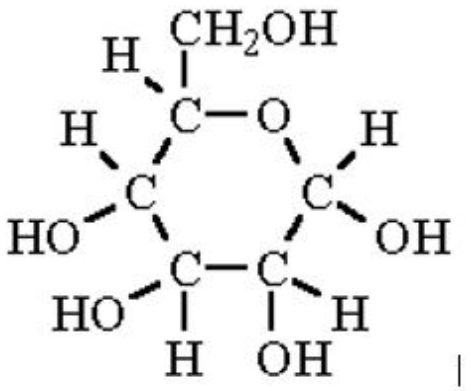

Figure 2.55. Molecular structure of glucose.

The products of carbohydrate digestion in the alimentary tract are almost entirely glucose, fructose, and galactose; glucose comprises 80%. After absorption, most of the fructose and galactose is rapidly converted into glucose in the liver. Within hepatocytes, enzymes are available to promote interconversions among the glucose, fructose, and galactose; of particular relevance, these cells contain a large amount of glucose phosphatase, so that the glucose-6-phosphate can be degraded to glucose and phosphate, and the glucose then being transported back into the blood. Within tissues, the glucose is broken down in a series of reactions releasing energy in the form of ATP. This occurs first through the anaerobic process of glycolysis, leading to the production of some ATP and pyruvate end-product. Under anaerobic conditions, pyruvate converts to lactate, while in aerobic conditions, the pyruvate enters the citric acid cycle to yield energy-rich electron carriers that help produce ATP.

Glucose reserves are stored as the polymer glycogen, which is present in the highest concentrations in the liver and muscle tissues. The regulation of glycogen, and, therefore, glucose, is controlled through the peptide hormones insulin and glucagon, both being produced in the pancreatic Islet of Langerhans, glucagon in from alpha-cells, and insulin from beta-cells. Through processes of signaling cascades regulated by these hormones, glycogen is catabolized liberating glucose, promoted by glucagon in times of fasting, or synthesized further consuming excess glucose, facilitated by insulin in energy-rich times.

There are different types of glucose transporters in the body, with two main forms, the sodium-dependent transporters (SGLTs) and sodium-independent transporters (GLUT). The former rely on the active transport of sodium across the cell membrane, which then diffuses down its concentration gradient along with a molecule of glucose. The sodium-independent transporters do not rely on sodium and transport glucose using facilitated diffusion.

There are many hormones involved with glucose homeostasis. Insulin decreases blood glucose through increased expression of the transporter GLUT4, increased expression of glycogen synthase, inactivation of phosphorylase kinase and decreasing the expression of rate-limiting enzymes involved in gluconeogenesis. Glucagon increases blood glucose through increased glycogenolysis and gluconeogenesis. Somatostatin decreases blood glucose levels through local suppression of glucagon release and suppression of gastrin and pituitary tropic hormones. Cortisol increases blood glucose levels via the stimulation of gluconeogenesis and through antagonism of insulin. Epinephrine increases blood glucose levels through glycogenolysis and increased fatty acid release from adipose tissues. Thyroxine increases blood glucose levels through glycogenolysis and increased absorption in the intestine. Growth

hormones promotes gluconeogenesis, inhibits liver uptake of glucose and stimulates thyroid hormone. ACTH stimulates cortisol release from adrenal glands and stimulates the release of fatty acids from adipose tissue.

Clinically relevant conditions are associated with either high or low blood glucose levels. Hyperglycemia can cause pathology, both acutely and chronically. Diabetes mellitus I and II are both disease states characterized by chronically elevated blood glucose levels. Type I, most often presenting in the young, is associated with genetic, environmental, and immunological factors, resulting from autoimmune destruction of pancreatic beta-cells. Type II is associated with conditions such as obesity and results from peripheral insulin resistance owing to metabolic dysfunction. In both cases, the result is inappropriately elevated blood glucose, which causes pathology by a variety of mechanisms such as osmotic damage to peripheral nerves, oxidative stress and non-enzymatic glycation These mechanisms lead to a variety of clinical manifestations including peripheral neuropathies, poor wound healing, retinopathy, coronary artery disease, and chronic kidney disease. High blood sugar levels can also lead to acute pathology, especially with type II diabetes, known as a hyperosmolar hyperglycemic state which occurs when there is a severely elevated blood glucose level resulting in elevated plasma osmolality. The high osmolarity leads to osmotic diuresis and dehydration, possibly causing motor abnormalities, CNS dysfunction and other conditions. Hypoglycemia is most often seen in diabetic patients secondary to glucose-lowering drugs and with the interruption of the patient's usual diet. Clinical symptoms may be neuroglycopenic, owning to a direct effect on the CNS, leading to fatigue, behavioral changes, seizures, coma, and death.

Galactose

Galactose is a carbon 4 (C-4) epimer of glucose; as a monosaccharide, it can be directly absorbed and enter the bloodstream to fuel energy production, glycosylation and other metabolic functions. Galactose, like all other aldohexoses, has two enantiomers: D-galactose or L-galactose, the latter not naturally occurring in higher living organisms as it cannot be further metabolized within the cell. Galactose can occur in linear or cyclic form, the six-carbon form of the latter being galactopyranose. In milk, galactose is present in the form of lactose, a disaccharide formed by a glycosidic linkage between one molecule of galactose and one of glucose. This glycosidic bond can be hydrolyzed by the enzyme lactase, present in the apical surface of the intestinal microvilli. Most of ingested galactose is retained in the liver, while small amounts reach other organs, such as the brain or the mammary glands where it is used for the synthesis of amino acids or for the production of lactose, respectively. In the liver and skeletal muscles, most of the galactose is metabolized *via* three main routes: the Leloir pathway, where it flows into glycolysis or glycosylation, conversion to galactonate that is further metabolized to enter the pentose phosphate pathway, and reduction to galactitol which is excreted with urine. Galactose plays an important role in protein glycosylation, a post-translational covalent modification essential for functional maturation of many proteins and lipids. The glycoconjugates have many effects within and outside cell, including intracellular signaling, modulation of protein and lipid functions, cell-cell communication, cell-matrix adhesion, glycocalyx formation, and immune response modulation.

Starch

Starch is a natural polysaccharide, in its native form consisting of glucose molecules. It can occur in two forms, amylose, an amorphous linear polymer and amylopectin, a crystalline, branched chain. Some modified starches, such as cross-linked high amylose corn starch, may be used as functional ingredients in sustained release formulations of drugs to maintain therapeutic concentration over extended periods of time; these are usually in the form of microspheres or hydrogels, with good gel-forming ability, biodegradability and biocompatibility.

Cellulose
Cellulose is a linear polysaccharide, with many hundreds of kinked D-glucose units. It is a critical structural component of the cell wall of green plants and many forms of algae and is present in some bacteria. In humans it is a non-digestible constituent of insoluble dietary fiber but otherwise has limited interest for medical technologies.

2.3.1.2.2 Glycoproteins and proteoglycans
Glycoproteins are proteins to which carbohydrates (especially, glucose, mannose, fucose, acetylgalactosamine, acetylglucosamine, acetylneuraminic acid, and xylose) are covalently bound through glycosidic bonds. The carbohydrate content is typically 50-60% by weight. Glycoproteins in the human body in the body are important parts of the membranes and Golgi apparatus in cells and serve as the cellular recognition molecules such as receptors and adhesion molecules. The process of binding the carbohydrate to the protein is called glycosylation, which usually occurs post-translationally with most RER synthesized proteins undergoing glycosylation. N-glycosylation involves the attachment to nitrogen on the amine side chain of asparagine, and O-glycosylation is the attachment of glycans to oxygen on serine and/or threonine.

Glycoproteins have many functions in the body, including structure and involvement in immunity. Mucins are glycoproteins that are secreted into the mucus of respiratory and digestive tracts, with gel-like characteristics that allow effective lubrication. Specific glycoproteins (and glycolipids) that are present on red blood cell surfaces determine blood group type, for example A-oligosaccharide for the A group, B-oligosaccharide for the B group, both A & B oligosaccharides for the AB group, and the absence of both A & B for O group. Some hormones are glycoproteins including follicle-stimulating, which as a gonadotropin hormone is involved in development, growth, puberty, and reproduction.

Proteoglycans are considered as a subclass of glycoproteins, essentially composed of a protein core with one or more covalently attached glycosaminoglycan chains, having just 10-15% carbohydrate; they have long unbranched chains with disaccharide units as repeating structures, compared with the glycoproteins that are short highly branched glycan chains with no repeating units. Proteoglycans can be divided into several categories, including heparin sulfate, chondroitin sulfate, and keratan sulfate. They are found mainly in connective tissues.

2.3.1.2.3 Glycosaminoglycans
Glycosaminoglycans (GAGs), or mucopolysaccharides, are negatively-charged polysaccharides composed of repeating disaccharide units. They are present in every mammalian tissue and covered a wide range of functions determined by their molecular structure. They are involved in cell hydration and structural scaffolding, and in cell signaling. There are several primary groups of GAGs, with a classification are classified based on the disaccharide units; these are heparin/heparan sulfate, chondroitin sulfate, dermatan sulfate, hyaluronic acid and keratan sulfate.

Hyaluronic acid
Hyaluronic acid (Hyaluronan or HA) is a non-sulphated GAG, composed of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine linked by a glucuronide β (1→3) bond. Although with a simple composition, without variations in its sugar composition or without branching points, HA has a variety of physicochemical properties, forming specific stable ternary structures in aqueous solutions. HA polymers exist with many possible configurations and shapes, depending on their size, salt concentration, pH, and associated cations. Unlike other GAG, HA is not covalently attached to a protein core, but it may form aggregates with proteoglycans. HA encompasses a large volume of water, giving solutions high viscosity.

HA is widely distributed in prokaryotic and eukaryotic cells. For humans, it is most abundant in the skin, the vitreous of the eye and synovial fluid, but is also found in skeletal tissues, heart valves, the lung, the aorta, the prostate, and corpora cavernosa. It is produced in many cell types but primarily by mesenchymal cells. HA is synthesized by specific enzymes, HA synthases. These are membrane bound; HA is synthesized on the inner surface of the plasma membrane and extruded through pore-like structures into the extracellular space. HA has a variable turnover rate. HA has a half-life of 3 to 5 min in the blood, less than a day in the skin and 1 to 3 weeks in the cartilage. HA is degraded into fragments of varying size by hyaluronidases by hydrolyzing the linkages between N-acetyl-D-glucosamine and D-glucuronic acid residues in HA. HA can also be degraded non-enzymatically by a free-radical mechanism in the presence of reducing agents such as ascorbic acid, thiols, ferrous, or cuprous ions, a process that requires the presence of molecular oxygen.

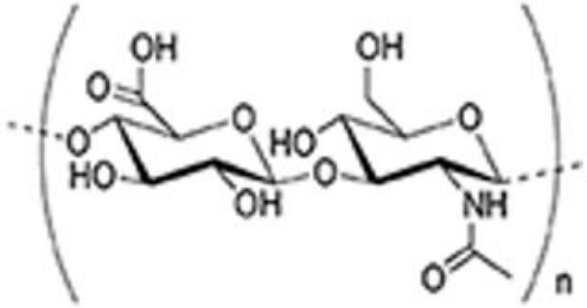

Figure 2.56. Molecular structure of hyaluronic acid.

HA of high molecular size, in excess of 1,000 kDa, is present in intact tissues and is both antiangiogenic and immunosuppressive; smaller polymers are distress signals and potent inducers of inflammation and angiogenesis. Synthesis increases during tissue injury and wound healing. HA also provides the framework for blood vessel formation and fibroblast migration, that may be involved in tumor progression. General functions include hydration and joint lubrication.

A variety of proteins that bind HA, called hyaladherins, are widely distributed in the ECM, the cell surface, and the cytoplasm; those that attach HA to the cell surface constitute HA receptors. The most prominent among these receptors is the transmembrane glycoprotein CD44 that occurs in many isoforms. CD44 is found on virtually all cells, except red blood cells, and regulates cell adhesion, migration, lymphocyte activation and homing, and cancer metastasis. The interactions between HA and the receptor for HA-mediated motility (RHAMM) control cell growth and migration by a complex network of signal transduction events and interactions with the cytoskeleton.

Skin HA accounts for 50% of total body content. HA in the dermis regulates water balance, osmotic pressure and ion flow and functions as a sieve, excluding certain molecules, enhancing the extracellular domain of cell surfaces and stabilizing skin structures by electrostatic interactions. Elevated levels of HA are synthesized during scar-free fetal tissue repair. Dermal fibroblasts provide for the synthesis of dermal HA and has been the target for pharmacologic attempts to enhance skin hydration. However, exogenous HA is cleared from the dermis and is rapidly degraded.

Heparin
Heparin and heparan sulfate are linear, acidic glycosaminoglycans. In humans, they are found on the surface of cells and in the extracellular matrix, attached to a protein core. They are built up of linear chains of repeating disaccharide units consisting of a glucosamine and uronic acid. They are structurally diverse giving rise to a varied range of physiological processes that they modulate; they have been shown to interact with a large number of proteins, regulating activities such as cell proliferation, inflammation, angiogenesis, and viral infectivity. Heparin is a well-known anticoagulant drug; a distinct pentasaccharide sequence within heparin chains is crucial for binding and activating antithrombin, leading to accelerated inhibition of the coagulation cascade.

For therapeutic uses, three forms of heparins have been available, unfractionated heparin (UFH), low molecular weight heparin (LMWH) and synthetic ultra-low molecular weight heparin, (U)LMWH. Commercially available UFH may be isolated from porcine intestinal mucosa or lung or bovine intestine, but the manufacturing process of pharmaceutical-grade heparins is complex, including the purification, isolation and drying of highly charged heparin molecules from other GAGs. Quality control was very difficult due to highly variant chain lengths and sulfation patterns, impeding purification. This led to the use of an adulterant heparin product undetected by standard quality control, causing anaphylactic reactions and several deaths worldwide, leading to the need for synthetic heparins.

LMWHs are produced from UFH by chemical and enzymatic depolymerization, yielding smaller polysaccharide fragments. Due to low affinity for plasma proteins, endothelial and blood cells, LMWHs show better subcutaneous bioavailability and longer half-life, enabling application once or twice a day. Causing fewer adverse reactions than UFH, LMWHs have been recommended for prophylaxis and therapy of thromboembolic events. As just one third of individual heparin molecules show anticoagulant properties, further attempts have been made to biochemically synthesize only ULMWHs or pentasaccharides.

Heparin and heparan sulfate are also able to modulate several cellular functions, and it is within these processes that applications in regenerative medicine are being considered. During ECM assembly, fibronectin fibrils interact with proteins and growth factors; fibronectin has binding sites for heparan sulfate and heparin, influencing fibronectin conformation and regulating the presentation of growth factors at the cell surface. Heparin may differentially influence cell adhesion *via* neural cell adhesion molecules, selectins and integrins. Heparin and heparan sulfate also significantly regulate genes involved in cell adhesion and proliferation in human bone marrow-derived stromal cells. Heparin also plays a pivotal role in cell proliferation, acting as co-factor for growth factors of the FGF family, the transforming growth factor (TGF)-beta superfamily, vascular endothelial growth factors (VEGFs) and platelet derived growth factors (PDGFs).

2.3.1.3 Lipids

Lipids are a structurally diverse group of molecules, best defined as substances that are soluble in non-polar organic solvents but not in water. They are the structural components of cell membranes, and serve as energy stores, sources of signaling molecules and in protein recruitment. Lipids can be hydrophobic or amphiphilic (i.e., having a polar water-soluble group attached to a water-insoluble hydrocarbon chain). With a hydrophilic headgroup at one end and a hydrophobic region at the other end allows these regions to align with each other to form a monolayer. Two monolayers can come together to form a membrane, with the water-soluble hydrophilic region facing the aqueous environment. Thus, the plasma membrane of cells separates the inside of a cell from the extracellular space, whilst intracellular membranes form organelles that can segregate different metabolic functions. The main lipids present in mammalian cell membranes are of three types, glycerolipids, sphingolipids and sterols. Glycerolipids use glycerol as their

backbone whilst sphingolipids use sphingosine, an amino alcohol, and sterols have four rings arranged in a specific molecular configuration with a hydroxyl group at one end and a short hydrocarbon side-chain at the other. A few lipids have extremely important functions. Lipids used for energy storage are the glycerolipid, triacylglycerol, in which the three hydroxyls of the glycerol backbone have fatty acids attached to them. Lipids that contain a phosphate are known as phospholipids and these are the main lipids of the membrane together with cholesterol.

2.3.1.3.1 Triacylglycerol

Nearly all the commercially important fats and oils of animal and plant origin consist almost exclusively of triacylglycerols; these are chemically inert, highly hydrophobic substances that have a high energy density. They consist of the trihydric alcohol glycerol, esterified with long-chain fatty acids. The more abundant animal triacylglycerols are depots fats (from adipose tissue) or milk fats, where their main functions are to store of energy and, especially in the liver, influence cellular metabolism. The subcutaneous fats also help to insulate animals.

2.3.1.3.2 Cholesterol

Cholesterol is a structural component of cell membranes and serves as a building block for synthesizing various steroid hormones, vitamin D, and bile acids. Besides their structural role providing stability and fluidity, cholesterol also plays a crucial role in regulating cell function. It is a 27-carbon compound, the structure of which has a hydrocarbon tail, a central sterol nucleus made of four hydrocarbon rings, and a hydroxyl group. The hydrocarbon tail and the central ring are non-polar and do not mix with water. Cholesterol is therefore packaged with apoproteins in order to be circulated in blood as a very low-density lipoprotein (VLDL), which therefore contains triglycerides, cholesterol, and phospholipids. Degradation of triglycerides in VLDL results in smaller low-density lipoproteins (LDL) that are rich in cholesterol, which also travel through the blood circulation, being delivered to the peripheral tissues where it is recognized by the LDL receptors on the cell membranes and is endocytosed. Some high-density lipoproteins (HDLs) also carry cholesterol from the peripheral tissues to the liver in a reverse transport mechanism to remove excess.

Cholesterol has several biological functions, with a major role in cellular homeostasis, maintaining cellular membrane rigidity and fluidity. It acts as a precursor to bile acids and assists in steroid and vitamin D synthesis; all classes of steroid hormones, glucocorticoids, mineralocorticoids, and sex hormones, are derivatives of cholesterol. Bile is a watery mixture of several compounds, phosphatidylcholine and conjugated bile salts and acids being the most important. Some of the bile is synthesized from cholesterol in the liver. Vitamin D3 (cholecalciferol) undergoes hydroxylation by 25-alpha hydroxylase to form 25-hydroxycholecalciferol in the liver from lipid-soluble compounds with a 4-ringed cholesterol backbone. Vitamin D plays an integral role in the terminal differentiation of hypertrophic chondrocytes and calcification of the bone matrix. With respect to sex hormones, cholesterol is converted to pregnenolone, which is then oxidized and isomerized to progesterone and further modified by various hydroxylation reactions to other steroid hormones, including cortisol, androgens, and aldosterone. Aldosterone acts on the renal tubules, stimulating potassium excretion and an increase in blood pressure. The androgens, specifically testosterone, estrogens, and progestins, are responsible for sexual differentiation, libido, spermatogenesis, and ovarian follicle production.

As well as being vital for many cellular functions, elevated levels of cholesterol can have serious consequences. Gallstones can form if there is either a bile salt deficiency or excess cholesterol secreted into the bile; there must be a proper balance of bile salts, cholesterol, and phospholipids, otherwise cholesterol will precipitate. In pathologic states of hypercholesteremia, gallstones are often formed, leading to cholecystitis. Dietary and lifestyle factors may result in an increased level of circulating LDL lipoproteins. When levels are pathologically high, it deposits in the arterial wall and oxidizes. Macrophages engulf these oxidized LDL particles, triggering the activation of cytokines, growth factors,

leukocytes, with neovascularization and smooth muscle cell proliferation, causing the formation of atherosclerotic plaques and coronary artery disease. Familial hypercholesterolemia is an inherited condition involving mutations in genes such as the LDL receptor gene, where LDLR can no longer clear the LDL from the blood, resulting in high cholesterol levels in the blood which, if left untreated, can again cause coronary heart disease. It should be mentioned here that there is one class of drug, that can be used in the treatment of hyperlipidemia and hypercholesterolemia. Statins are reversible competitive inhibitors of HMG-CoA reductase, a rate-limiting enzyme in cholesterol synthesis; statins may be used to stop *de novo* synthesis in the liver.

2.3.1.3.3 Phospholipids

Phospholipids, which are ubiquitous to all tissues, are essential components of cell membranes. They consist of a hydrophilic head group and a hydrophobic tail, which gives them their amphiphilic properties. There are several groups of phospholipids. Glycerophospholipids contain two fatty acid molecules esterified in the different positions of the glycerol moiety. The simplest glycerophospholipid is phosphatidic acid, others being named after the hydrophilic residue/group attached to the phosphate group, specifically, ethanolamine, inositol, serine, and choline. The most biologically important phospholipids are, therefore, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and phosphatidylcholine. In addition, lysophospholipids are phospholipids whose fatty acid chain has been removed from either of the positions. Sphingolipids contain the long-chain amino alcohol sphingosine instead of glycerol, esterified to a fatty acid and a phosphate group. Sphingomyelin consists of sphingosine with a choline molecule, being found in high quantities in brain and neural tissues membranes.

The hydrophilic head and the hydrophobic tail, and the resulting amphiphilic nature, create a lipid bilayer that controls the assembly of cell and organelle membranes, forming selectively permeable barriers, that allow for low membrane permeability for cellular constituents. The lipid bilayer organization provides a matrix in which the membrane-integral proteins are embedded; the integrity and function of the cellular and subcellular membrane systems depends on the integrity of their phospholipid structures.

Phosphatidic acid[146]constitutes the essential building block from which most glycerophospholipids are synthesized, therefore being associated with the regulation of a large number of intracellular signaling pathways and cellular functions. Phosphatidic acid is composed of a glycerol backbone esterified with two fatty acyl chains and with a phosphate group. The latter confers its specific features compared to the other diacyl–glycerophospholipids, this small anionic phosphate headgroup provides a combination of unique cone-shaped geometry and negative charge, enabling it both to interact with different enzymes to regulate their catalytic activity and to affect membrane geometry by creating local negative curvatures.

*Phosphatidylethanolamin*e[147] is a non-bilayer forming phospholipid containing a small polar head group diameter in proportion to its fatty-acid chains. Its intrinsic properties induce the formation of hexagonal phases within the membrane, promoting membrane fusion and fission events, protein integration and conformational changes. It is the second most abundant phospholipid in human cells, comprising 15–25% of the total. It is functionally associated with protein biogenesis, oxidative phosphorylation, autophagy, membrane fusion, mitochondrial stability, and is a precursor of other lipids.

[146] Tanguy E, Wang Q, Moine H, *et al,* Phosphatidic acid; From pleiotropic functions to neuronal pathology, *Frontiers in Cellular Neurosciences,* 2019; 13(2). doi:10.3389/fncel.2019.00002.

[147] Calzada E, Onguka O and Claypool SM, Phosphatidylethanolamine metabolism in health and disease. *International Review of Cell and Molecular Biology,* 2016;321:29-88. doi:10.1016/bs.ircmb.2015.10.001.

Phosphatidylinositol-based lipids (phosphoinositides) play important roles in numerous aspects of intracellular signaling and in the anchoring of glycosylphosphatidylinositol-linked proteins to the membrane. These lipids function as important virulence factors and modulators of the host immune response in some infections.

Phosphatidylserine is essential for healthy nerve cell membranes and myelin. The body can make phosphatidylserine, but most of what it needs comes from foods; it is frequently taken as a supplement since exogenous phosphatidylserine is absorbed efficiently, crossing the blood–brain barrier, and slowing or reversing structural deterioration in nerve cells. It supports cognitive functions, relating to both short-term and long-term memory, the ability to focus attention and concentrate. It also supports locomotor functions, especially rapid reactions and reflexes.

Phosphatidylcholine is a major constituent of cell membranes and is responsible for the maintenance of cell-membrane integrity through control of information flow that occurs within cells from DNA to RNA to proteins, the formation of cellular energy and intracellular communication or signal transduction. Decreased cell-membrane fluidization and breakdown of cell- membrane integrity, as well as impairment of cell-membrane repair mechanisms, are associated with several disorders, including liver disease, neurological diseases, various cancers, and cell death. Phosphatidylcholine is also the major delivery form of the essential nutrient choline, which is a precursor in the synthesis of the neurotransmitter acetylcholine. Phosphatidylcholine is absorbed into the mucosal cells of the small intestine, following some digestion by the pancreatic enzyme phospholipase, producing lysophosphatidylcholine, reacylation of which takes place in the intestinal mucosal cells, reforming phosphatidylcholine, which is then transported by the lymphatics in the form of chylomicrons to the blood. Phosphatidylcholine is transported in the blood in various lipoprotein particles, VLDL, LDL and HDL. Phosphatidylcholine may be indicated to help restore liver function in a few disorders, including alcoholic fibrosis, and possibly viral hepatitis. There is some evidence that it may be useful in the management of Alzheimer's disease and some other cognitive disorders.

2.3.1.4 Concluding Comments about Large Molecules

I have dealt with the matter of large molecules at some length because of their huge significance in the functioning of the human body; if we fail to understand this, we do not have much chance in effectively reconstructing that body when things go wrong. There is, however, little to write about in respect to the spirituality and artistic sides of large molecules, primarily because we cannot see them. It is difficult for a philosopher or artist to get excited about things they cannot see, and probably which they cannot understand anyway. Such restrictions do not tend to get in the way of poets, who can write about anything, whether they see or understand it, or not. The following is a poem about myosin[148]:

> Smaller than the wavelength of light
> like a drunk walking
> a length of pipe, myosin staggers battered
> by jet-fast random atoms
>
> each raised leg
> flung into all
> possible positions
> in chaos-driven steps

[148] John Donlan, "The Muscle Motor Molecule Myosin", originally appearing in 'Rescaling CanLit: Global Readings': Special Issue of Canadian Literature, 2019; 238:65.

until one fits and locks;
work gets done;
heat-maddened water spins CANDUs;
I stumble into poetry;

house finches sing
delirious with lust without a plan
feathered heads are turned and hearts
are lifted and again it's spring.

We can easily pontificate about and visualize tendons, but not the collagen molecules within them. The same goes for beautiful hair and nails but not the keratin molecules, about the not-so-beautiful bundles of fat, but not the fatty acid molecules, and so on. What we can do is use molecular simulations and computer modelling to get some idea what they might look like, and some of the images make good art:

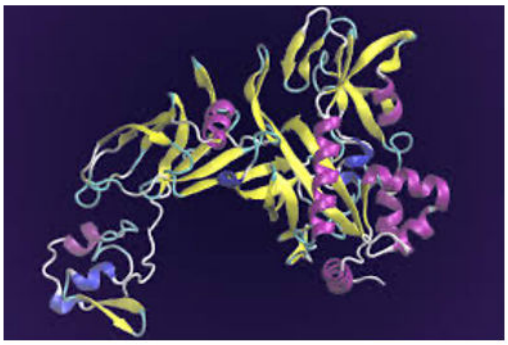

Figure 2.57 (a) Supercomputing-based models of protein structure, ORNL US Dept. Energy.

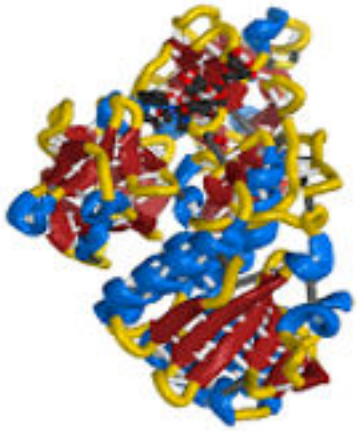

Figure 2.57 (b) Molecular model of glycosidase carbohydrate, US NIH.

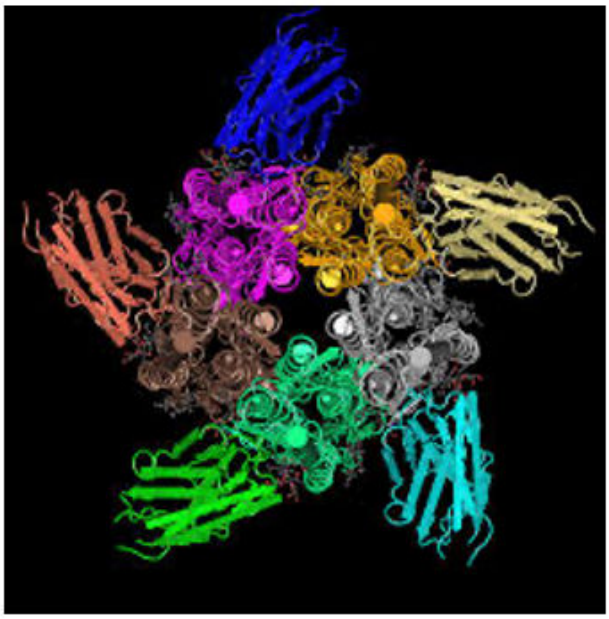

Figure 2.57 (c) Cryo-electron micrograph of receptors in lipid bi-layer, US National Center for Biotechnology Information

Some artists can use their skills to represent microscopic images. In the images below, neural stem cells are seen on the left where a red dye stains proteins most commonly seen in astrocytes. These have been represented in the image on the right using fabrics such as yams, organza and beads.

Figure 2.57 (d) Cells and fabrics, In Cell Press, Image by Maria Morrell, University of Michigan, Art Quilt by Bunnie Jordan, Fiber Artists.

2.3.2 Elements, Compounds, Minerals and Small Molecules

We now move to smaller molecules that are essential in the human body. I was initially going to use the simple heading 'Small Molecules" for this section, but that term is now widely associated with small molecule drugs in pharmaceutics, and its use would have been confusing. I am essentially covering those substances that have not been included in the preceding section and will not be included in the following section on genetic materials. It is a somewhat diverse group, and it is best not to even try to find common grounds upon which to logically combine them in one section.

2.3.2.1 Water

In many societies water has two meanings. Almost universally, it represents sacred values such as purity, fertility, beauty and life itself; on the other hand, it is equated with mysterious, dangerous and chaotic experiences. We could consider this from a very simple perspective, in that within all these original societies there were four common denominators, fire, earth, air and water, so it is not surprising that they, collectively or individually were the bases of all aspects spiritual. However, many people have considered this dualistic meaning of water, making it especially useful for explaining the incongruous aspects of life. Water imagery is widely used in the context of the health of the human body and of social groups and beyond, becoming a symbol of potency and of the outward influence of humans[149].

These dualistic meanings have influenced how people shape similarities and differences between themselves and others, water becoming the essence of social being, by either closeness or connectivity, but also to emphasize difference, as the ritual practices of washing and bathing determine social differences in Indian society, or how mineral water spas represented social capital for fashionable eighteenth century aristocrats in parts of Europe.

As far as the human body is concerned, we all need for water to survive. Health is maintained by absorbing water from the outside and by excreting what is excessive back into that outside world. The flow of water between that world and the inner body is used to transfer meanings and values from one experience to another. Drinking water restores the physiological balance, but cultural attitudes and behaviors are represented by how water is drunk. Similar considerations apply to how the body is washed with water and how excess water is excreted. Thus, different health-related aspects of water can be identified throughout history and are present in all cultures.

Water is the major chemical component of the human body. Approximately 65% of total body water is intracellular, and 35% extracellular water[150]. The actual volume in a healthy adult fluctuates because of ongoing physiological processes, the consumption of food and drink, differences in climate, salt intake and level of physical activity. The volume is further affected by disease, especially renal insufficiency, along with diabetes, liver disease, cancer, and heart disease. Healthy adult men, on average, consistently have larger amounts of total water than women in view of their larger size and muscle mass. mean values range from 35 to 45 liters in men 25 to 33 liters in women. The level starts to decrease around middle age in men and women and is rapid in women after approximately 60 years of age. Total water typically comprises 50 to 60% of adult body weight, but with a range from 45 to 75% depending on gender, age and levels of leanness or fatness.

[149] Verouden NW and Meijman FJ, Water, health and the body: the tide, undercurrent and surge of meanings, *Water History*, 2010; 2:19–33, doi:10.1007/s12685-010-0019-y.
[150] Chumlea, WC, Guo SG, Zeller CM *et al*, Total body water for white adults 16 to 64 years of age: The Fels Longitudinal Study, *Kidney International*, 1999;56:244-52.

2.3.2.1.1 Functions of water in the human body

It may seem self-evident why water is needed in the human body, but it is worth just mentioning these functions here. First, it is vital for the transport of substances throughout the body, including nutrients and waste products; mush of this is achieved within blood, which is 83% water. Water plays an essential part of maintaining pH within very tight limits in the various compartments of the body, and also maintaining proper electrolyte balance. It actively participates in many chemical reactions in the body, maintaining many of the biological pathways discussed in this book. From a physico-chemical perspective, it serves to moisten many tissues, for example in the mouth, nose and eyes, it facilitates shock absorption in the musculoskeletal system and the processes of temperature regulation.

2.3.2.2 Oxygen

If it is obvious that the body needs water to survive, the absolute requirement for oxygen is even more apparent; whilst the human body can survive for some days without taking in new supplies of water, even if in discomfort, the limit to survival without intake of oxygen is measured in minutes. Oxygen participates in a multifaceted integrated system, involving transport from lungs (and a few other sources) to mitochondria within cells, energy generation involving oxygen and the role of oxidative phosphorylation, the generation of oxygen-related species, such as reactive oxygen species within tissues, and their role in the transcription of many genes, and the influence of either insufficient or excess of oxygen in tissues.

2.3.2.2.1 Oxygen transport in blood

This has been discussed to some extent in previous sections on the lungs / respiratory system and hemoglobin, so will only be mentioned briefly here. For this discussion, blood may be considered as being composed of two phases, plasma and red blood cells. The fractional volume of blood occupied by red cells is the hematocrit, which is around 40% in females and 45% in males. Oxygen in the blood is either dissolved in plasma (about 2%) or reversibly bound to hemoglobin (about 98%). Thus, at physiological partial pressure of oxygen, only a small amount is dissolved in plasma since it has such a low solubility. Interestingly, at elevated partial pressures, for example when breathing pure oxygen or during hyperbaric oxygenation, the dissolved form of oxygen can become significant. The hemoglobin molecule has four binding sites for oxygen molecules. Thus, each gram of hemoglobin can combine with 1.39 ml of oxygen. In 100 ml of blood, there is about 15 g of hemoglobin, so that 100 ml of blood has the capacity to bind about 20 ml of oxygen. This quantity is the oxygen-binding capacity of blood, which is proportional to the hematocrit.

2.3.2.2.2 Oxygen transport in tissues and oxidative phosphorylation

In tissues, oxygen diffuses down its concentration gradient from high to low concentrations, and is delivered to cells, where it will act as the terminal electron acceptor in generating adenosine triphosphate through oxidative phosphorylation. Electron transport and oxidative phosphorylation are critical activities of proteins in the inner mitochondrial membrane, serving as the major source of cellular energy. During this process, electrons derived from NADH and $FADH_2$ (discussed later in this section) combine with oxygen, and the released energy drives the synthesis of ATP from ADP. The transfer of electrons from NADH to oxygen is strongly energy-yielding, but to be usable, this energy must be produced gradually, by the passage of electrons through a series of carriers, within the electron transport chain, which are organized into four complexes in the inner mitochondrial membrane. A fifth protein complex then serves to couple the energy-yielding reactions of electron transport to ATP synthesis. Complex I consists of nearly 40 polypeptide chains; the electrons are transferred from NADH to flavin mononucleotide and then to coenzyme Q, a small, lipid-soluble molecule that carries electrons from complex I to complex III. Here, electrons are transferred from cytochrome *b* to cytochrome *c*, a peripheral membrane protein which carries electrons to complex IV, cytochrome oxidase where they are finally transferred to oxygen.

2.3.2.2.3 Oxygen metabolites

The diatomic molecule oxygen contains two uncoupled electrons and can therefore undergo reduction, yielding several different oxygen metabolites, which are collectively called Reactive Oxygen Species or ROS. They are produced in aerobic environments through a variety of mechanisms, which include electron "leakage" during biologic oxidations, action of flavin dehydrogenases, and specific membrane associated secretion, as well as by physical activation of oxygen by irradiation, e.g. ultra violet sun-light. The most common ROS are superoxide anion, hydrogen peroxide and hydroxyl radical. These metabolites are all highly reactive and have many effects in most organisms, either directly or through conversion into other derivates, notably nitric oxide-derived radical (NO) or Reactive Nitrogen Species (RNS). Depending on their tissue concentration ROS can either exert beneficial effects, such as the control of gene expression and mitogenesis, or damage cell structures, including lipids, proteins and nucleic acid, potentially leading to cell death. The inappropriate metabolism of oxygen may thus be toxic to cells and tissues and contribute to the etiology of a wide variety of diseases. At present cardiovascular and neuronal diseases are possibly linked to the imbalance of radical formation and antioxidant defense. ROS contribute to responses to inflammation and bacterial invasions.

2.3.2.2.4 Hypoxia and hyperoxia

In many organisms, a lack of oxygen supply or an excessive oxygen consumption may result in insufficient oxygen levels for maintaining normal cellular function, defined as hypoxia[151]. Generally, hypoxia involves a relatively low (e.g., < 2%) oxygen content compared to normal status in an organ, tissue, or cell. It can occur with a continuous lack of oxygen for a short time (acute hypoxia such as ischemia) or long period (chronic hypoxia, as with chronic kidney disease or cancer). In humans, central and peripheral chemoreceptors sense the reduction of oxygen tension and send signals to respiratory centers, where processes are initiated to increase pulmonary ventilation and cardiac output. If this does not occur efficiently, there are pathological impacts in several human conditions including cancer, cardiovascular, chronic kidney and metabolic diseases, preeclampsia, and endometriosis.

For example, hypoxia is a common feature in the pathophysiology of several cardiovascular disorders. Reduced left ventricular systolic function after myocardial infarction results in insufficient oxygen, potentially leading to heart failure. Even if the ejection fraction is preserved, this can eventually lead to systemic and pulmonary hypertension. The latter also associates with other hypoxic pulmonary diseases, including chronic obstructive pulmonary disease and obstructive sleep apnea syndrome, and promotes inflammation and atherosclerosis, which itself can increase the risk of myocardial infarction and stroke. The thickness of the arterial wall may be a factor that causes hypoxia in the intima, reducing the perfusion of the tissue, and further stimulates pro-atherosclerotic processes, such as inflammation, lipid synthesis, and angiogenesis.

On the other hand, breathing oxygen at higher-than-normal partial pressure leads to hyperoxia possibly causing oxygen toxicity. Acute toxicity, involving very high concentrations for a short time, generally induces central nervous system effects, while chronic toxicity mainly has pulmonary effects. Severe cases of oxygen toxicity can lead to cell damage and death; those at particular risk include hyperbaric oxygen therapy patients, patients exposed to prolonged high levels of oxygen, premature infants, and underwater divers. Such exposures can cause oxidative damage to cell membranes, leading to the collapse of the alveoli in the lungs. Symptoms include chest pain and dyspnea secondary to tracheobronchitis. Early signs in the CNS are quite variable but twitching of perioral and small muscles of the hand are common. If exposure to oxygen pressures is sustained, tinnitus, dysphoria, nausea, and convulsions can develop.

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2.3.2.3 Cations

It was difficult in the last two sections separating small from large molecules. It is much more difficult in separating the entities when they are at atomic or ionic dimensions; indeed, there are several, equally relevant, ways in which this could be done, in terms of cations, anions, electrolytes and so on. I have chosen to start with cations, where the vast majority are based on metallic elements. Within this group, we must consider factors such as 'normal' concentrations in the body, essentiality status, toxicity, speciation, dangerous levels, either high or low, and medical technology / pharmaceutics relevance.

2.3.2.3.1 Characteristics of metallic elements

There are 118 known elements, of which 98 occur naturally. Of these, 32 exist in pure form, the others only in combination with other elements. I am choosing about 50 of these elements (all metallic which form cations) that are of some relevance to the physiology of the human body and/or its reconstruction and provide some basic facts. They are given in increasing order of atomic number (in brackets). Note the following:

- Speciation refers to the different oxidation states that can exist with the element in the human body, which is of great significance with respect to toxicology[152],
- Essentiality referred to as major, minor, uncertain, non-essential,
- Clinical applications given for the elements and/or its compounds,
- Tissue levels are representative of data in literature, given in most relevant units,
- MRL – Minimal Risk Level,
- NOAEL – No Observed Adverse Effects Level,
- mEq/L – milliequivalents, in blood,

Lithium (3)[153,154]

Essential – minor
Clinical – use in therapy for bipolar disorders
Maintenance reference serum level, 0.5-0.8 mmol/L
Toxicity serum level (renal) > 1.2 mmol/L

[152] Templeton DM, Speciation in Metal Toxicity and Metal-Based Therapeutics, *Toxics,* 2015; 3(2): 170–86. doi:10.3390/toxics3020170.

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Beryllium (4)[155,156]

Non-essential
Clinical – used in imaging equipment, but no *in vivo* applications
Maintenance reference serum level, not detectable, or < 0.3 μg/L
Toxicity urine level (respiratory, sensitivity, carcinogenesis) > μg/L

Boron (5)[157]

Non-essential, but considered a 'micronutrient'
Clinical – antimicrobial, dietary supplement
Maintenance reference serum level, 15-80 μg/L
Toxicity (respiratory) MRL, oral, 0.2 mg/kg/day; NOAEL, inhalation, 0.8 mg/m^3

Sodium (11)

Essential, electrolyte
Normal levels in adult, 135-145 mEq/L
Hyponatremia, <135 and hypernatremia, >145 both dangerous[158]

Magnesium (12)[159]

Essential
Clinical – many pharmaceutical applications; dietary supplement; implantable devices[160]
Equilibrium levels (mmol/kg wet weight), bone: 43.2, muscle: 9, soft tissue: 8.5: red cells: 2.5, serum: 0.85.
Hypomagnesaemia, <0.75 mmol/L and hypermagnesaemia, >2 mmol/L, many symptoms

Aluminum (13)[161,162]

Non-essential
Clinical – non-active component of drugs, vaccine adjuvant, as alloying addition for implanted devices, as oxide ceramic in implants and dentistry
Normal levels in liver and bone, 6 μg/g dry weight, elsewhere <1 μg/g
Many forms of toxicity associated with pro-oxidant actions, neurotoxicity at >10 μg/g. Alzheimer patients ten time more aluminum in brain than controls.

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[160] Tan J and Ramakrishna S, Applications of magnesium and its alloys: A review. *Applied Sciences*, 2021;11:6861. doi:10.3390/app11156861.

[161] Exley C, Human exposure to aluminum, *Environmental Sciences: Processes and Impacts,* 2013;15:1807-16. doi:10.1039/C3EM00374D.

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Potassium (19)

Essential
Clinical- drug to control low plasma levels
Normal levels in adult, 3.5-5.2 mEq/L
Hypokalemia <2.5 , hyperkalemia >5.5, both dangerous

Calcium (20)

Essential
Clinical – drug to control low levels, calcium phosphates used in orthopedic and dental surgery
Normal levels in adult, 2.2 – 2.6 mEq/L
Hypocalcemia <2.2, hypercalcemia >2.6, associated with overactive parathyroid, kidney disease and osteoporosis

Scandium (21)[163,164]

Non-essential, no known biological role
No clinical application, no recent evidence of human toxicity

Titanium (22)[165]

Beneficial for crops[166] but not essential for humans[167]
Clinical – metal used for implantable devices, oxides used as non-active components of drug and in sun- screens
Normal serum levels in adults, 30-60 μg/L
No systemic levels for toxicity, occasional case reports of allergies. Generally non-toxic

Vanadium (23)[168] Speciation: IV/V

Possible minor essential element[169]
Clinical - alloying addition in medical technology, use in some drugs, e.g., for diabetes, and dietary supplements
Normal serum levels in adults, 1 nmol/L[170]
No systemic levels for toxicity[171], some GI effects with diabetic drugs

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Chromium (24) Speciation: III/VI

No conclusive evidence for essentiality
Clinical - questionable dietary supplement, alloying addition in medical devices
Normal serum levels in adults, < 5 μg/L; deficiency at < 0.15 μg/L
Cr (III) mildly toxic, mean lethal dose 2,000-3,000 mg/kg
Cr (VI) high toxic, carcinogenic, mean lethal dose, 50 mg/kg[172]

Manganese (25) Speciation: II/III/IV

Essential[173]
Clinical – in some drugs and dietary supplements
Normal serum levels in adults, 0.5 μg/L accumulates in basal ganglia
Toxicity rare[174], pathological threshold of concern in brain, 150 μM.

Iron (26) Speciation: 0/II/III

Essential
Clinical – component of steels and other alloys for implanted devices, oxide used in drug delivery and imaging contrast agents
Normal serum levels in adults, 15-30 μmol/L,
Both acute and chronic iron overload conditions, severe at 90 μmol/L.

Cobalt (27)[175] Speciation: II/III

Essential, component of Vitamin B12
Use in alloys for implantable medical devices
Normal serum levels, adult males 25 μg/L and females, 70 μg/L
Toxicity variable, systemic and local, extreme cases after hip replacement, serum at 1000 μg/L [176]

Nickel (28) Speciation: II/IV

Minor essential
Use in alloys for implantable medical devices
Normal serum levels in adults, 0.2 μg/L
Variable toxic effects, hypersensitivity, carcinogenicity, teratogenesis, difficult to quantify[177]

Copper (29) Speciation: 0/I/II

Essential, enzyme co-factor
No significant medial uses
Normal serum level in adults, 2 μmol/L, of ceruloplasmin, 5 μmol/L
Rare cases of hypocuremia[178], some rare diseases of copper overload, e.g., Wilson's disease[179]

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Zinc (30) Speciation: 0/II
Essential, protein binding[180]
No significant medical uses
Normal serum level in adults 800 μg/L[181]
Rare cases of mild toxicity

Gallium (31)
Non-essential
^{68}Ga used in imaging
Very little reliable information on tissue levels, <700 μg total in average person[182]
Toxicity very rare[183]

Germanium (32)
Non-essential
No significant medical applications, some doubtful food supplements, organo-germanium compounds used in chemotherapy[184]
Very little information on tissue levels
Toxicity very rare, but concern over food supplements[185]

Arsenic (33) Speciation: III/IV
Non-essential
Arsenic trioxide used to treat blood cancer[186]
Normal whole blood level in adults 2 μg/L, total content in land mammals, 0.1-0.4 ppm
Highly toxic, both acute and chronic, arising from ingestion[187]

Selenium (34)[188] Speciation: II/IV/VI
Essential, as selenoproteins

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Clinical – few applications but some uses as dietary supplement
Normal whole blood levels, 50 μg/L
Generally non-toxic, but acute poisoning with supplements, with serum levels >750 μg/L[189]

Strontium (38)
Non-essential
Clinical -history of dietary supplements; component of some apatite ceramics for bone reconstruction and in magnesium implantable alloys
Normal serum levels in adult humans, 30 μg/L
Little evidence of significant human toxicity

Yttrium (*39)*
Non-essential
Clinical – used in imaging systems, as additive in alloys and ceramics for implantable devices[190].
Normal serum levels in adult humans, 35 μg/L
Little evidence of significant human toxicity

Zirconium (40)
Non-essential
Clinical – oxide ceramic used in orthopedic implants and in dentistry Sodium zirconium cyclosilicate used in therapy of hyperkalemia[191]
Normal serum levels in adults, 0.1 μg/L
Not considered toxic for humans or significant industrial health hazard

Niobium (41)
Non-essential
Clinical – occasional use as alloying addition in some orthopedic applications
Normal serum levels in adults very low, probably < 0.1 μg/L
Not considered toxic for humans or significant industrial health hazard

Molybdenum (42) Speciation: II/III/IV/VI
Highly essential in plants, minor essential for humans[192]
Clinical – occasional use as alloying addition in some orthopedic applications
Normal serum levels in adults, 0.5 μg/L
Limited evidence, generally not considered toxic to humans

Rhodium (45)
Non-essential
Clinical – no significant uses
Normal serum level in adults, 1ng/L
Limited evidence, some metallo-complexes bind to DNA[193]

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Palladium[194] (46) Speciation: II/IV
Non-essential
Clinical – component of some dental alloys and implantable electrodes
Normal serum level in adults 10 ng/L
Limited evidence, not significant toxicity but some hypersensitivity[195,196]

Silver[197] (47) Speciation: 0/I/II
Non-essential
Clinical – antibacterial agent in wound dressings and catheter coatings[198]
Normal serum levels in adults 2 μg/L
Toxicity controversial, low with metallic silver[199]. Overload gives skin color (argyria) but no serious effects.

Cadmium (48)
Non-essential
Clinical – no applications
Unknown, usually below limit of detection, 0.3 μg/L
Highly toxic[200]

Tin (50) Speciation: II/IV
Non-essential to humans but considered essential to some species (e.g., fish)
Clinical – very few significant applications
Normal serum levels in humans, 0.5 μg/L
Limited evidence, some toxicity with organo-tin complexes

Barium (56)
Non-essential
Clinical – radiopacity (as sulfate) used in imaging and as filler in polymeric devices
Very variable barium serum levels in humans, usually 10-20 μg/L
Rare cases of poisoning with some soluble salts but generally low toxicity[201]

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Gadolinium (64)
Non-essential
Gadolinium-based contrast agents used in MR imaging
Normally undetectable
Free gadolinium ions extremely toxic, highly localized[202]

Tantalum (73)
Non-essential
Clinical – used (including porous forms[203]) for orthopedic and dental implant applications.
Little data, serum levels less than 1 ng/L
Normally low toxicity, but rare localized effects around implants

Tungsten (74)
Non-essential
Clinical – occasional use as alloying addition in implantable devices
Little data, serum levels less than 0.5 μg/L
Toxicity controversial[204], but not high

Iridium (77)
Non-essential
Iridium-192 used in brachytherapy. Used with platinum as alloy for electrodes and leads
Little data, serum levels less than 0.1 μg/L
Limited evidence, generally not considered toxic to humans

Platinum (78) Speciation: II/IV
Non-essential
Platinum alloys used as conducting components in implantable devices. Cisplatin used for chemotherapy
Normal levels in adult serum 1 μg/L
Metal has low toxicity, but accumulation of complexes considered to have high toxic potential[205]

Gold[206] (79) Speciation: 0/I/III
Non-essential
Clinical – dental crowns, electrodes, nanoparticles for imaging and drug delivery, gold complexes as drugs for rheumatoid arthritis
Normal serum levels in adults 0.5 μg/L
Very low toxicity of metal to humans[207]

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[205] Zhang Y, Zheng J, Jiang Y, *et al,* Neglected, drug-induced platinum accumulation causes immune toxicity, *Frontiers in Pharmacology*, 2020;11:1166. doi: 10.3389/fphar.2020.01166.
[206] Balfourier A, Kolosnjaj-Tabi J, Luciani N, *et al*, Gold-based therapy: From past to present, *Proceedings of the National Academy of Sciences*, 2020;117(37):22639-48. doi:10.1073/pnas.2007285117.
[207] Lansdown ABG, Gold: human exposure and update on toxic risks, *Critical Reviews in Toxicology*, 2018;48(7):596-614. doi:10.1080/10408444.2018.1513991.

Mercury (80) Speciation: 0/I/III
Non-essential
Clinical -previous use in dental amalgam
Normal level in human blood < 10 μg/L
Highly toxic, both metal vapor and organic compounds[208]

Lead (82) Speciation: II/IV
Non-essential
Clinical – none in Western medicine
Very variable blood levels, depends on environmental exposure, between 1 and 50 10 μg/L
Very toxic, no level is assumed to be safe

2.3.2.3.2 Comments on metallic cation levels

Metallic elements clearly have some very important roles in human biology; it is crucial that these roles are respected when any medical technology that introduces metals into the body is used. As noted above, several of these elements are essential to human life, some do not appear essential but are well tolerated within a wide concentration range, while some have significant toxic effects under certain circumstances. Among the more obvious effects of metal ion excess in tissues are the generation of free radicals, especially reactive oxygen species, which can lead to damage of lipids in cell membranes, the inactivation of enzymes and effects on proteins (leading to misfolding and conformational change), all affecting cellular function, as well as effects on nucleic acids and regulatory molecules that could be associated with carcinogenesis. There have been some well-documented clinical scenarios in reconstructive techniques where metal ions introduced into the body have caused significant adverse events; there are few common pathways with these examples, so they will be dealt with in later chapters in relation to their respective technologies and devices.

2.3.2.4 Inorganic Compounds and Minerals

As well as the cations discussed in the previous section, the body contains several types of anion that are crucial to performance, and indeed life itself. The most important, of these, and the molecules and compounds that they form are mentioned here.

2.3.2.4.1 Phosphates

Phosphates constitute about 1% of the total body weight, which decreases with age. Normal serum phosphate levels are between 2.5 and 4.5 mg/dL. Around 85% of the total phosphate is found in in the bones and teeth; elsewhere phosphates are present in cell membranes, nucleic acids, intracellular signaling proteins and phosphate esters. Within the bones and teeth, it is largely present as hydroxyapatite , with a smaller amount as amorphous calcium phosphate.

Although the crystal structure appears fairly straightforward, that is not the case since the ionic constituents are present non stoichiometrically, where there are variations in their ratios and, equally importantly, other ions can substitute for the calcium, phosphate and hydroxyl ions to some extent. In naturally occurring hydroxyapatite, these ions include CO^{2-}_3, Na^+, Mg^+, Fe^{2+}, F^-, Zn^{2+} and some

[208] Ye B-J, Kim B-G, Jeon M-J, *et al*, Evaluation of mercury exposure level, clinical diagnosis and treatment for mercury intoxication, *Annals of Occupational and Environmental Medicine*, 2016;28:5. doi:10.1186/s40557-015-0086-8.

silicates[209]. Most intervening ions substitute for the calcium, but carbonates and silicate can substitute for the phosphate, while the fluoride and chloride substitute for the hydroxyl. The substituted ions all appear to have some role in bone and tooth development and metabolism. As will be seen in later sections, the possibility of replicating the beneficial effects of substituent ions, especially strontium and the carbonates / silicates, has been investigated extensively in bone regenerative materials. Radioactive strontium, accidentally released from nuclear plants such as Chernobyl and Fukushima, finds its way into the hydroxyapatite of developing bones and teeth[210].

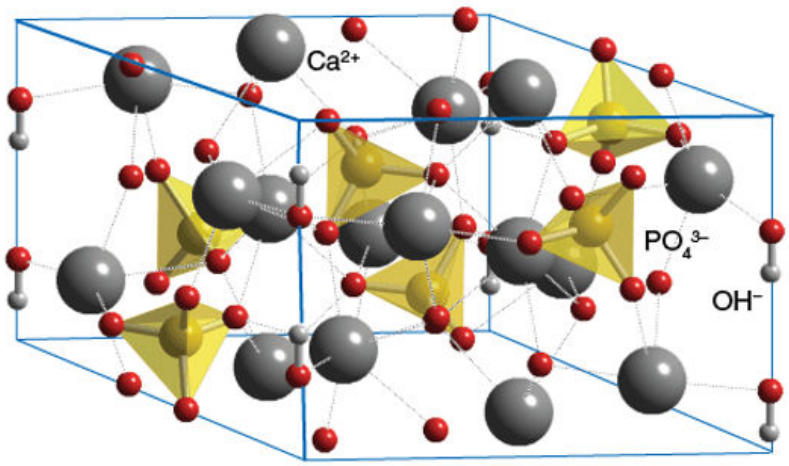

Figure 2.58. Crystal structure of calcium hydroxyapatite, $Ca_5(PO_4)_3\,OH$.

Apart from this role in hydroxyapatite structures in mineralized tissues, phosphates are involved in several cellular functions. It is an important component of the lipid bilayer of cell membranes and in DNA, RNA and protein structures. Serum levels are tightly regulated since hyperphosphatemia is associated with chronic kidney disease and cardiovascular morbidity, while hypophosphatemia can lead to rickets and osteomalacia.

Mention must be made here of the related group of molecules based on the naturally-occurring inorganic pyrophosphates and synthetic derivatives of the bisphosphonate group[211]. The inorganic pyrophosphate has two phosphate groups linked by esterification; it is present in many tissues and is capable of inhibiting calcification by binding to hydroxyapatite crystals, this being one of the processes that regulate bone mineralization. Synthetic bisphosphonates have common features with the inorganic pyrophosphate but contain a central nonhydrolyzable carbon atom, usually with a hydroxyl group attached to this carbon atom. The phosphate groups provide the bisphosphonates with a strong affinity for hydroxyapatite, which

[209] Ratnayake JTB, Mucalo M and Dias GJ, Substituted hydroxyapatites for bone regeneration: A review of current trends, *Journal of Biomedical Materials Research, Part B*, 2017;106B:1285-99. doi:10.1002/jbm.b.33651.
[210] Takahashi A, Chiba M, Tanahara A, *et al,* Radioactivity and radionucleotides in deciduous teeth formed before the Fukushima-Daiichi Nuclear Power Plant accident, *Scientific Reports,* 2021;11:10335. doi:10,1038/s41598-021-89910-0.
[211] Drake MT, Clarke BL and Khosla S, Bisphosphonates: Mechanism of action and role in clinical practice, *Mayo Clinic Proceedings*, 2008;83(9):1032-45.

is enhanced by the attraction between the hydroxyl group and calcium. By a combination of these attractive forces, bisphosphates have a high specificity for bone, and when used as pharmaceutical agents, have significant roles in the treatment of disorders such as osteoporosis and bone metastases[212]. Several molecules can be used, one of the most effective being alendronate, which is very effective in minimizing bone resorption. This is a powerful focus in reconstructing the skeletal system.

2.3.2.4.2 Chlorides

The chloride anion, Cl^- has a negative charge and, in the human body, is commonly associated with the cations sodium, Na^+ and potassium K^+ . As such, the chloride constitutes an important electrolyte, where it maintains fluid balance, oxygen transport and the acid-base balance. The chloride ion moves freely across cell membranes; within cells it associates with potassium, while in extracellular fluid it associated with sodium. Potassium concentrations are about 30 times higher inside than outside cells, while sodium concentrations are more than 10 times lower inside than outside cells. The concentration differences between potassium and sodium across cell membranes create an electrochemical gradient, the membrane potential. This is maintained by ion pumps in the cell membrane, especially the Na^+/K^+ ATPase pumps that use ATP (see below) to pump sodium out of the cell in exchange for potassium. Their activity accounts for 20%-40% of the resting energy expenditure in a typical adult. Tight control of cell membrane potential is critical for muscle contraction, nerve impulse transmission and cardiac function.

Since sodium is the primary determinant of extracellular fluid volume, several regulatory mechanisms work by adjusting the sodium content. In the circulatory system, baroreceptors sense changes in blood pressure and send excitatory or inhibitory signals to the nervous system to affect sodium regulation by the kidneys. Several regulatory mechanisms control sodium transport in various segments of the nephron, including the proximal and distal convoluted tubules, the thick ascending limb of the loop of Henle, and the collecting duct. For example, in response to a significant decrease in blood volume, the kidneys release renin into the circulation. An enzyme that splits angiotensin I from angiotensinogen, which is further split into a smaller peptide, angiotensin II, by angiotensin converting enzyme (ACE), which stimulates the constriction of small arteries, resulting in increased blood pressure. Excessive intakes of sodium chloride lead to an increase in extracellular fluid volume. Hypernatremia, defined as serum sodium concentrations >145 mM, is much less common than hyponatremia and rarely caused by excessive sodium intake, generally developing from excess water loss (e.g., burns), or reduced water intake.

Most intracellular potassium is contained within muscle cells, so the total body potassium is roughly proportional to lean body mass. Potassium is a major determinant of intracellular osmolality and relatively small alterations in serum potassium concentration can have significant clinical manifestations, including muscle weakness and cardiac arrhythmias. Several factors shift potassium in or out of cells; insulin moves potassium into cells so that high concentrations of insulin lower serum potassium levels. Low concentrations of insulin, as in diabetic ketoacidosis, cause potassium to move out of cells, thus raising serum potassium. Beta-adrenergic agonists, especially selective beta 2-agonists, move potassium into cells, whereas beta-blockade and alpha-agonists promote movement of potassium out of cells. Acute metabolic acidosis causes potassium to move out of cells, whereas acute metabolic alkalosis causes potassium to move into cells.

2.3.2.4.3 Spirituality aspects of chlorides

Chlorides, especially sodium chloride, are obviously very important entities that control many aspects of human life; they are also very simple entities which have direct counterparts in the living world outside of human bodies. The significance of sodium chloride, that is, salt, in human diets has been known for a

[212] Russel RGG and Rogers MJ, Bisphosphonates: From the laboratory to the clinic and back again, *Bone,* 1999;25:97-106.

long time. It is not surprising, therefore, that salt has figured prominently in many philosophical and religious writings and practices.

There are many references to salt in the Bible, although they are of a contradictory nature. Salt had been used in many cultures as a seasoning or preservative of food, a disinfectant, a component of ceremonial offerings, and as a unit of exchange. The Hebrews made it clear that salt was an important part of ancient Hebrew religious sacrifice, as in Leviticus (2:13) "*And every oblation of thy meat offering shalt thou season with salt; neither shalt thou suffer the salt of the covenant of thy God to be lacking from thy meat offering: with all thine offerings thou shalt offer salt*". Newborn babies were rubbed with salt, for its antiseptic qualities as well as a way of proclaiming blessings into their lives. In Matthew (5:13), with reference to the Sermon on the Mount, Jesus tells his disciple, "*You are the salt of the earth*" but goes on to say "*but if salt has lost its taste, how shall its saltiness be restored? It is no longer good for anything except to be thrown out and trampled under people's feet.*" In Colossians (4: 6) the apostle Paul tells Christians, "*Let your conversation be always full of grace, seasoned with salt*".

The fascination with salt in both Old and New Testaments has been attributed to Jews who lived next to the Dead Sea, a salt lake that was the main source of salt to all neighboring communities. The Old Testament refers to the use of salt to consecrate land that had been used for battle to the Lord. This ritual being referred to as "salting the earth." Much attention has been paid to the role of salt in the Old Testament story of Sodom and Gomorrah, and the fate of Lot's wife (Genesis 13, 5-13;18,20-37 and 19,1-29, especially verse 26). Lot and his family lived in the land of Canaan. Abraham said to Lot that there was insufficient land enough here for all their animals, so Lot moved to the beautiful district of the Jordan and made their home in the city of Sodom. However, the people of Sodom were very bad, which upset both Lot and God. Finally, God sent two angels to warn Lot that he was going to destroy Sodom and the nearby city of Gomorrah They said to Lot, 'Hurry! Take your wife and your two daughters and get out of here!' One of the angels said: 'Run for your lives! Don't look back. Run to the hills, so that you won't be killed.' Lot and his daughters obeyed and ran away without stopping or looking back. But Lot's wife disobeyed; she stopped and looked back. The Bible takes up the story: "*The sun had risen on the earth when Lot came to Zoar. Then the Lord rained on Sodom and Gomorrah sulfur and fire from the Lord out of heaven. And he overthrew those cities, and all the valley, and all the inhabitants of the cities, and what grew on the ground. But Lot's wife, behind him, looked back, and she became a pillar of salt."*

Figure 2.59. Lot's wife and the Pillar of Salt.

The theological interpretation is that this shows us that God saves those who obey him, but those who do not obey him will lose their lives. But why salt? There have been many ideas about this, one of which is as follows, being based on the documented enjoyment of life in Sodom by Lot's wife. For thousands of years the primary function of salt was not as a seasoning but as a preservative. If Lot's wife had been allowed to flee the wickedness of Sodom to a better place, all the time harboring a love for her past wicked life, the evil of Sodom's wickedness would have gone with her, preserved deep within her, waiting to emerge and infect other lives. Rather than allow her to preserve the cherished memory, God preserves her as a pillar of salt. She becomes a memorial for the preservation of evil, a warning to all who might see her frozen in her half-turned gaze of longing.

The ambiguity surrounding the spiritual significance of salt can be seen in the history and myths of many cultures. In Mesopotamian mythology, Tiamat was a goddess of the sea, specifically personifying the salty sea, in contrast to Apsu who personified fresh water. Tiamat was seen as a monstrous embodiment of the chaos of primordial creation, seeking to de-stabilize the order of the universe. On the contrary, Huixocihuatl was a fertility goddess of the Aztecs and was patron of salt and salt waters. She was the older sister of the Tlaloques, the Rain Gods, including Tlaloc, the Lord of Celestial Waters; he tried to drown her in salt water after an argument, but she escaped and in so doing 'discovered the salt itself'. Salt was used to explain part of Buddha's philosophy. In teaching the monks, Buddha asked them, is someone puts a salt crystal into water contained in a small bowl, whether that water would be fit to drink. They responded by saying the water would taste salty and be unfit to drink. He then asked them if someone put a salt crystal in the waters of the River Ganges, would that make the river water unfit to drink. They replied that it would not because there is an immensely large quantity of water in the Ganges, making the salt unnoticeable and keeping the water safe. Buddha then responded 'In the same way, one person who commits a trivial unwholesome kamma will be born in hell, while another person who commits a similar minor unwholesome kamma will experience some ill effects in this life itself and will not be reborn in the hell' There have been many explanations and interpretations of this 'Simile of the Salt Crystal', especially in relation to what constitutes a trivial kamma (or action), but the effect of sodium chloride concentration is quite clear. Finally, it should be noted that in Japan, salt is considered sacred, being considered a preserver of purity (rather than of evil in the case of Lot's wife). Salt plays an important part in the purification rituals of Misogi and in Shinto rituals salt is offered to the kami, the mythological deities, in order to ward off evil spirits. During childbirth, salt is often used to purify the space, while it provides a 'force for life' in weddings, and as means to avoid spirits following funeral attended as they go home after the ceremony.

2.3.2.4.4 Fluorides

Fluoride is a reactive anion that is found in the environment and within the human body in combined states, including sodium fluoride and calcium fluoride, and minerals such as fluorite and fluorspar. The majority of fluoride in the human body is stored in bones and teeth, equilibrium amounts being less than 0.1 wt%[213]. Drinking water is the major source of fluoride intake[214]; it has been considered for many years that low fluoride levels are beneficial to the development and maintenance of bones and teeth, but excess levels can produce certain toxic manifestations. This has resulted in public health policies in some parts of the world to introduce fluoridation of drinking water where the levels were considered sub-optimal, that is less than around 0.5 mg/l[215]. Within the mineralized tissues, the fluoride ion substitutes for hydroxyl ions,

[213] Kim FM, Hayes C, Williams PL, *et al*, An assessment of bone fluoride and osteosarcoma, *Journal of Dental Research*, 2011;90(10):1171-6. doi:10.1177/0022034511418828.
[214] Perumal E, Paul V, Govindarajan V, *et al,* A brief review on experimental fluorosis, *Toxicology Letters,* 2013;223:236-51. doi:10.1016/j.toxlet.2013.09.005.
[215] Ozsvath DL, Fluoride and environmental health, *Reviews in Environmental Science and Biotechnology,* 2009;8:59-79. doi:10.1007/s11157-008-9136-9.

giving fluorapatites, $Ca_{10}(PO_4)_6F_2$ or $Ca_{10}(PO_4)_6$ OH,F, which increases structural stability and reduces mineral solubility. It is for the same reason that so many dental accessories such as toothpaste often contain sodium fluoride at levels around 1,000 ppm. Water fluoridation was not always popular and led to extensive public debate and the promotion of conspiracy theories[216]. There is no doubt that fluoride is effective in maintaining dental and skeletal health, but excessive fluoride intake can lead to manifestations of fluorosis[217]. It is an inhibitor of glycolysis and can cause cellular apoptosis. Chronic ingestion at high doses can induce dental fluorosis (including enamel pigmentation), decreased birth rates, kidney stones, and impaired thyroid function.

Figure 2.60. The water fluoridation controversy, from the Irish Times, 2013.

2.3.2.4.5 Bicarbonate

Bicarbonate, HCO_3^-, is a labile entity that undergoes pH dependent couple conversions, being in equilibrium with carbonate (CO_3^{2-}) and carbonic acid (H_2CO_3); the latter can reversibly disassociate into water and dissolved carbon dioxide, which is in equilibrium with gaseous carbon dioxide at interfaces in the lungs[218]. Bicarbonate is important in mitochondrial energy production and pH buffering throughout the body. The ion itself cannot pass through membranes and requires the support of transport proteins in order to fulfil its regulatory roles. There are several bicarbonate transport proteins, known as Solute Carriers (SLC) that engage in this process of transfer across plasma membranes. In addition, there are some anion channels that have permeability to bicarbonate and transport may be facilitated by certain n ZIP protein (Zrt/Irt-like proteins), which function in association with metal ions such as Zn^{2+}. Defective bicarbonate transport leads to diseases such as systemic acidosis, hypertension and kidney stone formation. There are also suggestions that alterations in this transport are important in some cancers.

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[216] McNeil DR, America's longest war: The fight over fluoridation, 1950-, *The Wilson Quarterly,* 1985;9(3):140-53.
[217] Nagendra AH, Bose B and Shenoy P, Recent advances in cellular effects of fluoride: an update on its signaling pathway and targeted therapeutic approaches, *Molecular Biology Reports*, 2021;48:5661-73. doi:10.1007/s11033-021-06523-6.
[218] Alka K and Casey JR, Bicarbonate transport in health and disease, *International Union of Biochemistry and Molecular Biology: Life,* 2014;66(9):596-615. doi:10,1002/iub.1315.

2.3.2.4.6 Silica, silicic acid and silicates

As noted elsewhere, silicon is a non-metallic element. It is unable to exist in its natural state due to its propensity to interact, under ambient conditions, with oxygen and water. In nature, as with the other major tetravalent element, carbon, it is able to form silicon-silicon, silicon-oxygen, silicon-nitrogen and silicon-carbon bonds. However, molecules featuring silicon instead of carbon have very different properties because of differences in atomic size and greater electronegativity[219].

Silica, SiO_2 occurs in nature in many forms, in both crystalline (e.g., quartz) and amorphous forms. Silicic acid, $Si(OH)_4$, is a water-soluble molecule, which can form silicates in combination with other minerals or metals; the silicate anions polymerize, with chains bonding to metal cations such as Fe^{2+}, Mg^{2+} and Ca^{2+}. These chains can be either single or double, the latter often having Al^{3+} or Na^{+} cations, forming highly colored minerals, for example tanzanite, which is the mineral zoisite, or calcium-aluminum-hydroxyl sorosilicate.

The silicic acid (or more specifically orthosilicic acid) is the main species found in human tissue, and the total amount of silicon contained in the body is around 1-2g[220]. The highest concentrations are in bone and some connective tissues, with good evidence that silicon has a positive effect on bone homeostasis. With solid silica, there are potential serious toxic side effects, the best-known being silicosis, a disease associated with chronic inhalation of silica or silicate dust. There are significant differences in the toxicity potential (including carcinogenicity potential) between the aggressive crystalline forms and amorphous particles[221] .

2.3.2.5 Molecules Associated with Messenger, Energy and Nutrition Functions

Although only marginally relevant to reconstruction processes, there are some other molecules that are essential to human life and should be mentioned briefly.

Chemical messengers are ubiquitous and may be included in several different classification compartments, but one group is of particular importance here; these are the hormones. The endocrine system is a network involving glands and organs that generates and delivers hormones to all parts of the body to regulate cellular mechanisms. There are more than 50 hormones in humans, but they are highly selective, and can only enter those cells that have the relevant receptors. The main endocrine glands are the hypothalamus, which essentially drives the whole of the endocrine system, the pituitary gland, which is linked to the hypothalamus and secretes hormones made by it (e.g. growth hormone), the thyroid gland which regulates metabolism, adrenal glands which are stress-responsive and regulate blood pressure and the salt / water balance, the pancreas which produces glucagon and insulin, and the reproductive gonads, producing estrogen or testosterone.

Several types of molecules are involved in energy production and transfer. Adenosine triphosphate (ATP) is a critical source of energy, used and stored within cells. It is a nucleoside triphosphate that consists of a nitrogenous base, a ribose sugar and three phosphate groups. Essential for its function is the provision of readily releasable energy in the bond between second and third phosphate groups. ATP is synthesized during cellular respiration within the mitochondria during glucose oxidation. Energy is released as the

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[219] Martin KR, The chemistry of silica and its potential health benefits, *The Journal of Nutrition, Health & Aging,* 2007;112):94-8.

[220] Gotz W, Tobiasch E, Witzleben S *et al,* Effects of silicon compounds on biomineralization, osteogenesis and hard tissue formation, *Pharmaceutics,* 2019;11:117. doi:10.3390/pharmaceutics11030117.

[221] O'Reilly KMA, Phipps RP, Thatcher TH, *et al,* Crystalline and amorphous silica differentially regulate the cyclooxygenase-prostaglandin pathway in pulmonary fibroblasts: Implications for pulmonary fibrosis, American *Journal of Lung Cellular and Molecular Physiology*, 2005;288:L1010-6. Doi:10.1152/ajplung.00024.2004.

ATP is oxidized, this being used for many intracellular functions, including muscle contraction and nerve impulse propagation. A second important molecule is NAD, nicotinamide adenine dinucleotide, which exists in the oxidized (NAD^+) and reduced (NADH) states. The intracellular pool of NAD^+ and NADH is regulated through a delicate balance between biosynthesis and breakdown by NAD^+ - consuming enzymes[222]. Because of the nature of this balance, this substance is a causative factor in several acquired and inherited disorders.

Vitamins are essential for many bodily functions, but generally cannot be synthesized within the body and reply on dietary or pharmaceutical sources. The amounts involved are usually small, their functions being regulatory with respect to some vital chemical reactions. Vitamins are either water-soluble or fat-soluble. The main water-soluble vitamins are B1 (thiamin), B2 (riboflavin), B6 (pyridoxine), B12 (cobalamin), niacin, folic acid, biotin and C (ascorbic acid); with the exception of Vitamin C, all of these water-soluble vitamins have a catalytic function, acting as co-enzymes in energy transfer or metabolism. Fat-soluble molecules are vitamins A (retinol), D (calciferol), E (tocopherol) and K; these vitamins are necessary for specific functions in highly differentiated tissues, including those in embryonic development.

2.3.2.6 Ectopic or Adventitious Mineral Deposits

Not all mineral deposits in the body are beneficial and some adventitious deposits can be exquisitely painful, with marked influences on quality of life. That this should occur should not be too surprising since some substances are present in highly balance equilibrium where perturbations may be seen, sometimes apparently at random, but often with genetic predisposition. Calcium is often the principal component here, and ectopic calcification is quite widely experienced, including in situations where reconstruction of the skeletal system is attempted. One particularly painful condition is calcific tendonitis, which affects the rotator cuff in the shoulder, these deposits containing poorly crystalline carbonated apatite associated with proteins[223] .

Calcium is also involved in some, but not all, kidney stones[224]. These stones, the cause of the condition known as urolithiasis, affects about 12% of the world population at some time in their life, Within the kidney, particularly in the loop of Henle, the urine concentrate consists of 95% water, 2.5% urea and 2.5% of a mixture of minerals, salts, hormones and enzymes. Many of these substances are reabsorbed within the proximal tubules and although the salt and acid-base balance is regulated, there is still significant quantities of minerals in the urine within the distal tubules; as a result, several different types of stone can form in several different locations within the urinary system. Calcium stones, principally composed of calcium oxalate and calcium phosphate, is the dominant renal stone, others being of magnesium ammonium phosphate (struvite), urate and cystine. The process of stone formation involves both nucleation and growth of crystals, the best method of avoidance being maintenance of high urinary volumes and resisting supersaturation with respect to the minerals. For those who are affected by stones, clinical attention has been focused on promotion of stone dissolution and relevant analgesia. Medical professionals have been aware of the basics of stone formation for thousands of years, and efforts in stone prevention and management have been prominent in, for example, traditional Chinese medicine for a long

[222] Zapata-Perez R, Wanders RJA, van Karnebeek CM, *et al,* NAD^+ homeostasis in human health and disease, *EMBO Molecular Medicine,* 2021;12:e13943. doi:10.15252/emmm.202113943.
[223] Darrieutort-Laffite C. Blanchard F and Le Goff B, Calcific tendonitis of the rotator cuff; From formation to resorption, *Joint Bone Spine,* 2018;85)60:687-92. doi:10.106/j.jbspin.2017.10.004.
[224] Alelign T and Petros B, Kidney stone disease: An update on current concepts, *Advances in Urology,* 2018;3068365. doi:10.1155/2018/3068365.

time[225]. These methods include uses of acupuncture and herbal remedies to increase urinary volume, increase magnesium excretion and inhibition of calcium oxalate nucleation.

Finally in this brief section I have to mention gout. This disease, referred to as 'gouty arthritis', was first identified by the Egyptians nearly five millennia ago, later being much discussed by Hippocrates[226]. Galen described the tophi features, where urate deposits caused swellings especially around some joints. The term itself (in full, '*gutta quam podagram vel artiticam vocan*t), arises from the Latin '*gutta*' which means '*drop*' in which one of the essential humors would flow, or drop, into a joint. Deposits were identified by the microscopy of van Leeuwenhoek, and they were identified as essentially of uric acid, in 1776. Uric acid is synthesized in the liver and intestines as the end-product of the metabolism or purines and also, endogenously, from dead and dying cells[227]. Most daily uric acid disposal occurs in the kidneys; however, humans do not possess uricase enzyme so they cannot oxidize uric acid to the more soluble form, allantoin, so that there is a tendency for precipitation of uric acid crystals in pre-disposed individuals. The susceptibility is partly genetic, but is largely controlled by diet, especially the avoidance of purine-rich foods. Often described as the 'illness of kings' because of the association with rich diet and lifestyle (King Henry VIII was notoriously affected), gout has been associated with many critical events in history, where key individuals were often incapacitated by the excruciating pain and unable to take part in decisions of immense importance. Benjamin Franklin was one such individual, and on one occasion he was moved to write the dialogue between himself and 'Madam Gout', part of which is reproduced here[228];

> FRANKLIN. Eh! Oh! Eh! What have I done to merit these cruel sufferings?
> GOUT. Many things; you have ate and drunk too freely, and too much indulged those of yours in their indolence.
> FRANKLIN. Who is it that accuses me?
> GOUT. It is I, even I, the Gout.
> FRANKLIN. What? My enemy in person?
> GOUT. No, not your enemy.
> FRANKLIN. I repeat it, my enemy; for you would not only torment my body to death, ruin my good name; you reproach me as a glutton and a tippler: now all the world, knows me, will allow that I am not the one or the other.
> GOUT. The world may think as it pleases; it is always very complaisant to itself, and sometimes to its friends; but I very well know that the quantity of meat and drink proper a man, who takes a reasonable degree of exercise, would be too much for another, who never takes any.
> FRANKLIN. I take -eh! Oh! – as much exercise – eh! As I can, Madam Gout.
> GOUT. I can scarcely acknowledge that as any objection. As to quacks, I despise them; they may kill you indeed, but cannot injure me. And, as to regular physicians, they are at last convinced that the gout, in such a subject as you are, is no disease, but a remedy and wherefore cure remedy? – but to our business, - there.
> FRANKLIN. Oh! Oh! – for Heaven's sake leave me and I promise faithfully never more to play at chess, but to take exercise daily, and live temperately.

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[225] Miyaoka R and Monga M, Use of traditional Chinese medicine in the management of urinary stone disease, *International Brazilian Journal of Urology*, 2009;35(4):396-405.
[226] Nuki G and Simkin PA, A concise history of gout and hyperuricemia and their treatment, *Arthritis Research and Therapy*, 2006;8 (S1):S1.
[227] El Ridi R and Tallima H, Physiological functions and pathogenic potential of uric acid, *Journal of Advanced Research*, 2017;8:487-93. doi:10.1016/j.jare.2017.03.003.
[228] Matthews B b(Ed), III – Dialogue Between Franklin and the Gout, Midnight 1780, In *The Oxford Book of American Essays*, 1914.

GOUT. I know you too well. You promise fair; but, after a few months of good health, you will return to your old habits; your fine promises will be forgotten like the forms of the last year' clouds. Let us then finish the account, and I will go. But I leave you with an assurance of visiting you again at a proper time and place; for my object is your good, and you are sensible mow that I am your *real friend.*

2.3.3 Genes, Chromosomes and Nucleic Acids

2.3.3.1 Overview

This section is concerned with the role of genetics in reconstructive technologies, covering aspects of disease or malformation causation and the management of genetic conditions before, during and after reconstruction. Genetics, the scientific study of genes and heredity, is, of course, a vast and complex subject and it is only possible (and relevant) to consider a select number of aspects. First, it is necessary to clarify the basic terms here, especially with respect to 'genetic' and 'heredity, and also the closely allied 'familial". These terms are often used interchangeably, but there are differences.

It is probably sensible to deal with these in reverse order. 'Familial' refers to features that routinely occur in members of a family. The weakest association here is simple familial tendencies, which may have little to do with biology and are mostly environmentally and socially determined. Familial traits are features that are shared and expressed by family members that are largely due to the inheritance of genes by offspring from their parents. Such traits are inherently genetically determined; these may have nothing to do with diseases or malformations but represent character and susceptibilities to lifetime events. Familial disease usually occurs in more individuals in a family than expected by chance; genetic and environmental factors may both play a role in determining susceptibility, but there is unlikely to be a single genetic cause. The term 'hereditary disease' is commonly used when the disease has a known, specific, genetic cause. 'Genetic diseases' are those conditions that are caused by one or more gene mutation; as we shall see, when mutations occur it does not automatically cause a disease but increases the risk the disease developing. It is important to note that not all genetic diseases are hereditary, some mutations are acquired, for example arising from smoking or radiation. In other situations, it is possible to acquire a hereditary disease from parents who never had the disease but were carriers of it.

The laws of inheritance are fairly well known, thanks to the pioneering work of Gregor Mendel in the nineteenth century[229]. This work was not directed towards the understanding of disease, but the recurrence of traits through different generations, established by observations on garden peas. His observations, however, led to discoveries of inheritance patterns of single gene diseases, since he established different patterns of gene segregation for selected traits, hence allowing determination of probabilities of recurrence in subsequent generations.

Since, as discussed later, most genes exist in one or more versions (referred to as alleles), individuals may carry a normal allele and/or a mutated disease-inducing allele, depending on the population frequency of the allele. A single-gene disease is usually inherited in one of several patterns depending on the location of the gene and whether one or two normal copies of the gene are needed for manifestation of that disease phenotype. The expression of the mutated allele, relative to the normal allele is characterized as dominant, co-dominant or recessive; there are five modes of inheritance for single-gene diseases: autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive and mitochondrial. Multiple affected family members may exhibit different levels of severity of a disease and genetic heterogeneity is

[229] Orel V, (translated by Finn S), *Gregor Mendel: The First Geneticist*, Oxford University Press, 1996, ISBN 10:0198547749.

common. Effects may be due to other genes influencing the gene phenotype, or different mutations in the same gene resulting in similar by not identical phenotypes.

In the **Autosomal Dominant** mode, each affected person has an effected parent, and it occurs in every generation. Examples are Huntington's disease, familial hypercholesterolemia, achondroplasia and neurofibromatosis.

In the **Autosomal Recessive** mode, both parents of an affected person are carrier, but it is not typically seen in every generation. Examples are sickle cell anemia, cystic fibrosis and phenylketonuria.

With **X-linked Dominant** cases, females are more frequently affected, but both males and females within the same generation may be involved. Examples are some forms of rickets and ornithine transcarbamylase deficiency.

On the other hand, the **X-linked Recessive** mode affects males most frequently, often in each generation, with important examples of hemophilia A and Duchenne muscular dystrophy.

The **Mitochondrial** mode can affect both males and females, although only being passed on by females, often appearing in every generation, examples including Kearns-Sayre syndrome, a rare but often fatal neuromuscular disorder.

Specific details of some of these genetic diseases that have relevance to reconstructive technologies will be given at relevant places in the text. It is obvious that since the concept of hereditary was not introduced into society until after Mendel's work was published in 1865, which more-or-less coincided with Charles Darwin's pronouncements about evolutionary biology, and since the term 'gene' was not introduced into scientific language until the ideas of the Danish pharmacist Wilhelm Johannsen were published in 1909, there is little historical perspective to the subjects of genetics and hereditary. It is true that Darwin did introduce the hypothesis of 'Pangenesis' in 1868[230] in which he proposed that all cells in an organism are capable of shedding minute particles, or gemmules, which migrated through the body and finally congregating in the gonads, then to be transmitted to the next generation. This really lacked scientific evidence and was largely dismissed but did pre-date the ideas of molecular particles based on DNA by some time.

It is obvious the philosophers in much older times wondered about how offspring's traits arose from their parents, both in human and animal species, and indeed within plants, and some early hypotheses followed a similar, if naïve, path to that of Darwin and others. Hippocrates, in the fifth century BC considered that physical substances originated from each part of the body, being concentrated in male semen and developing into a human within the womb. Aristotle[231] disagreed with much of this and considered inheritance from an observational point of view, namely:

an adequate theory of reproduction must explain eight different phenomena connected with inheritance:

1. *Offspring tend to resemble their parents more than other members of the same species.*
2. *Some offspring resemble the father while others resemble the mother, both (a) as a whole and (b) with respect to different parts,*
3. *Offspring tend to resemble their parents more than their ancestors,*

[230] Liu Y, A new perspective on Darwin's Pangenesis, *Biological Research*, 2008;83:141-9. doi:10.1111/j.1469-185X.2008.00036.x.

[231] Henry D, Aristotle on the mechanisms of inheritance, *Journal of the History of Biology*, 2006;39:425–55. doi: 10.1007/s10739-005-3058-y.

4. *Offspring tend to resemble their ancestors more than any chance individual of the same species,*
5. *Usually males resemble their fathers and females their mothers,*
6. *Nevertheless, sometimes males resemble their mothers while females resemble their fathers,*
7. *Offspring who fail to resemble either its parents or its ancestors may still look like a human being at any rate,*
8. *In extreme cases the offspring's observable form may fail to bear any likeness to a human being, at which point it is a monstrosity.*

Without any knowledge of the scientific bases of these observations, the subject of inheritance seems to have been placed in abeyance for many centuries. This was largely due to the existence of alternative perspectives based on the principles of biblical creationism. Although there are many interpretations of this concept, it is widely agreed that verses in the Epistle of St Paul the Apostle to the Romans, encapsulated these principle, especially Romans 4:16, in the King James version " Therefore *it is* of faith, that *it might be* by grace; to the end the promise might be sure to all the seed; not to that only which is of the law, but to that also which is of the faith of Abraham; who is the father of us all". The interpretation here is that all things in the universe were created and made by God; all things which now exist are sustained and ordered by God's providential care. Moreover, all theories of origin or development which involve evolution in any form are considered false. It is not surprising that in parts of the world where the Church held such sway over individuals, that academics and philosophers steered clear of challenging the creationism concept until such a time that scientific evidence was very persuasive. Even Immanuel Kant had difficulties. Throughout his life he was, in public, equivocal about the concept of epigenesis, that is the belief that an embryo progresses from an undifferentiated egg cell, and he seemed to juggle with different views of creation[232]; Kant contrasted two possible approaches to the idea of supernatural generation. Either God intervenes directly in the order of nature to create individuals at the moment of their generation, or God has created all individuals at creation and merely allowed them to unfold later. The field moved forward with the work of Jean-Baptiste-Pierre-Antoine de Monet, chevalier de Lamarck, more commonly known as Jean-Baptiste Lamarck, a French biologist who advocated that acquired characteristics are inheritable and he was an important forerunner of evolution theory. A century later, in the USA, Thomas Hunt Morgan provided evidence of the chromosomal bases of inheritance, receiving a Nobel Prize for this in 1933, while a few years later, Oswald Avery discovered DNA, demonstrating that this, and not any protein, was the carrier of genetic information. This led to the work of Watson, Crick and others on the DNA molecule, which is discussed in the next section.

2.3.3.2 Nucleotides and Nucleic Acids

2.3.3.2.1 Nucleotides

The starting point in the explanation of the molecular basis of genetics are the nucleotides.

A nucleotide has three parts. At the center is five-carbon sugar, which is attached to a nitrogen-containing ring, known as the base, and at least one phosphate group.

[232] Demarest B. Kant's epigenesis: specificity and developmental constraints, *History of Philosophy of Life Sciences*, 2017;39(1):3. doi:10.1007/s40656-017-0129-2.

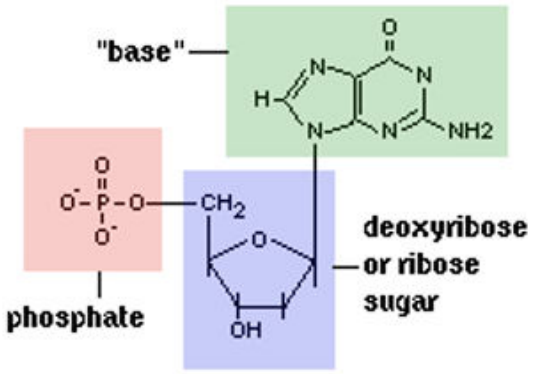

Figure 2.61. Structure of nucleotides.

The nitrogenous bases are of four principal types, adenine (A), guanine (G), cytosine (C), and thymine (T). A and G are purines since their structure contains two fused carbon-nitrogen rings; C and T, in contrast, are pyrimidines that have a single carbon-nitrogen ring. As noted below, there is another pyrimidine base, uracil (U). There are two forms of the five-carbon sugar ring, deoxyribose and ribose. They are quite similar in structure, but the second carbon of ribose bears a hydroxyl group, whereas the equivalent carbon of deoxyribose is linked to a hydrogen atom. Nucleotides may have a single phosphate group or a chain of up to three phosphates.

The nucleotides are essentially monomers, which may polymerize, to give polynucleotides. The monomers are joined together in a chain by ester linkages between the phosphoryl group of one nucleotide and the hydroxyl group of the sugar component of the next nucleotide.

There are two main forms of polynucleotides. Deoxyribonucleic acid, DNA, is made up of several monomeric units of nucleotides covalently bonded by 3′, 5′ phosphodiester linkages. This means that the 5′-phosphoric group of one nucleotide is esterified with the 3′-hydroxyl of the adjoining nucleotide. The nitrogenous base may be C, G, A or T. The two strands that make up the DNA form a helical structure wherein at the core the nucleobases are *complementarily* paired. The base pairing rules determine that adenine pairs with thymine whereas cytosine pairs with guanine. A hydrogen bond joins the two nucleobases. The two strands are *antiparallel*, which means they run in opposite directions to each other. Ribonucleic acid, (RNA) consists of a long linear chain of nucleotides, each unit being comprised of a ribose sugar, a phosphate group and a *nucleobase*. Ribose is a pentose sugar whose carbons are numbered 1′ through 5′. The phosphate group is connected to the 3′ of the ribose and the 5′ of another ribose. The nitrogenous base is connected to the 1′ of the ribose. The nitrogenous bases of the RNA are usually A, C, G and U. There are three major types of RNA, which are discussed below. DNA and RNA are natural polynucleotides, usually referred to as nucleic acids. DNA determines the sequence of amino acid in protein synthesis since it carries the genetic code with instructions or information in the genes.

2.3.3.2.2 DNA

Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus, but a small amount can be found in the mitochondria. The information in DNA is stored as a code made up of the four bases, A, G, C and T. DNA bases pair up with each other, A with T and C with G, to form base pairs. Nucleotides are arranged in two long strands that form a spiral double helix. Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of the bases determines the information available for building and maintaining an organism.

DNA replication is a process where the original (parent) strands of DNA in the double helix are separated and copied to produce a new (daughter) DNA molecule. In essence, each of the parent strands of the DNA molecule will be replicated by base pairing. The purine nucleobase (adenine and guanine) is paired with the pyrimidine nucleobase (cytosine and thymine). Specifically, the adenine will pair with thymine and guanine with cytosine. In the early stages of mitosis DNA is replicated and the process will culminate in the production of two cells containing identical copies of DNA.

DNA carries the genetic information that codes for a particular protein. During protein translation, the genetic code for a protein is first copied into the RNA (specifically, mRNA) a process called transcription which is assisted by the enzyme RNA polymerase. Although RNA polymerase traverses the DNA template strand from 3′ → 5′, the coding strand is usually used as the reference point. Hence, the process proceeds in the 5′ → 3′ direction, as in DNA replication and it uses base pairing to create an RNA copy containing U instead of T.

Mutations or errors occurring in the DNA are repaired by two major mechanisms, the direct reversal of the chemical process that caused the damage or the replacement of damaged nitrogenous bases. In direct reversal DNA repair mechanism, a template is not required and the change is superseded as the original nucleotide is restored. Repair is carried out by excising and replacing the damaged DNA with new nucleotides. The excision repair is of three forms: base-excision repair (where a single nucleotide change is recognized and subsequently excised by glycosylases), nucleotide excision repair (where multiple base changes are recognized and then cleaved by endonucleases), and mismatch repair.

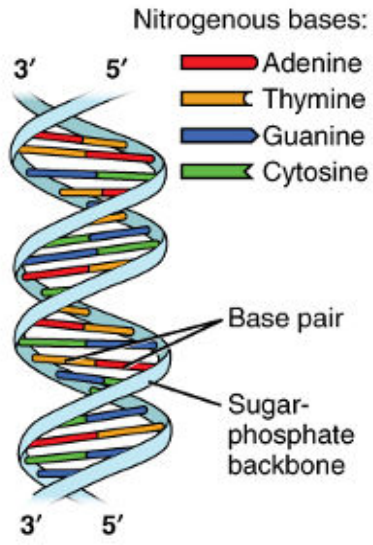

Figure 2.62. Structure of DNA.

DNA mutation leads to variability among species. Not all mutations lead to significant changes in the expression of the genetic code, but some produce improvement of a species, enabling it to acquire characteristics that could help it adapt better in its environment. However, there are also mutations in the genetic code that are pathological, leading to impaired protein function, metabolic disorders or physical deformities.

2.3.3.2.3 RNA

The main function of RNA is to generate the proteins necessary for cellular processes, *via* translation. Three main types of RNA are involved in protein synthesis, messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). RNA also serves as the primary genetic material for viruses. Other functions include RNA editing, gene regulation, and RNA interference, these processes being carried out by a group of small regulatory RNAs, which include small nuclear RNA, microRNA, and small interfering RNA.

mRNA is transcribed from DNA and contains the genetic blueprint to make proteins. In humans, a freshly transcribed RNA transcript is considered a pre-mRNA and needs to undergo maturation to form mRNA. A pre-mRNA contains non-coding (introns) and coding (exons) regions. During processing, the introns are spliced, and the exons are joined together. A 5' cap, 7-methylguanosine is added to the 5' end of the RNA transcript and the 3' end is polyadenylated, where a poly(A) tail, a sequence of adenine nucleotides, is added to the transcript. The 5' cap protects the mRNA from degradation, and the 3' poly(A) tail contributes to the stability of mRNA.

tRNAs are RNA molecules that translate mRNA into proteins. The function of tRNA is to carry amino acids on its 3' acceptor site to a ribosome complex with the help of aminoacyl-tRNA synthetase. The type of amino acid on a tRNA is dependent on the mRNA codon, which is a sequence of three nucleotides that codes for an amino acid. The anticodon arm of the tRNA is the site of the anticodon, which is complementary to an mRNA codon and dictates which amino acid to carry.

rRNA forms ribosomes, which are essential in protein synthesis. A ribosome contains a large and small ribosomal subunit. In humans, the 40S and 60S subunit form an 80S ribosome. The ribosomes contain an exit (E), peptidyl (P), and acceptor (A) site to bind aminoacyl-tRNAs and link amino acids together to create polypeptides.

RNA makes proteins using amino acids, of which there are 20 different types that constitute a protein's primary structure. When a ribosome binds to an mRNA transcript, it starts decoding the mRNA codons (A codon is a specific sequence of nucleotides on mRNA that corresponds to a specific amino acid or to a stop signal during protein translation) and recruits tRNAs with the encoded amino acid. Once a ribosome finishes reading the mRNA, the amino acid sequence will fold and form a protein.

With respect to the small regulatory RNAs, small nuclear RNAs (snRNA) are non-coding RNAs that are responsible for splicing introns, joining with proteins to form small nuclear ribonucleoproteins (snRNP). MicroRNAs (miRNA) are also non-coding RNAs that are mainly involved in gene regulation. Small Interfering RNAs (siRNA) are double-stranded, non-coding RNAs that inhibit gene expression through RNA interference, especially by degrading mRNA and preventing the translation of proteins.

One major concern with RNA is the possibility of mutations, which can be the result of defects in the RNA itself, in ribonucleoprotein complexes, or RNA binding proteins. Myotonic dystrophy, a neuromuscular disease, is caused by a CTG nucleotide repeat on the DMPK gene resulting in a pathogenic RNA gain-of-function. Spinal muscular atrophy may be caused by a mutation in splicing result in a mutated SMN2 gene. Other illnesses caused by RNA mutations include prostate cancer, Fragile X syndrome, and amyotrophic lateral sclerosis (ALS), discussed later in this book.

Finally, it should be noted that many viruses have RNA as their genetic material. This has enormous consequences in terms of diseases and vaccines, the significance of which will be seen later.

2.3.3.2.4 Discovery of the structure of DNA and its aftermath

In the early 1950s, the stage was set for one of the most remarkable, but controversial, discoveries in science. First, there was progress made by X-ray crystallographers in studying organic macromolecules. Then there was growing evidence supplied by geneticists that it was DNA, not protein, in chromosomes that was responsible for heredity followed by Chargaff's experimental finding that there are equal numbers of A and T bases and of G and C bases in DNA. Finally, Pauling discovered that the molecules of some proteins have helical shapes, using atomic models and knowledge of the possible disposition of various atoms.

Rosalind Elsie Franklin was born in London in 1920, the second of five children in a prominent Anglo-Jewish family. In 1938 she entered Newnham College, one of two women's colleges at Cambridge University, majoring in physical chemistry, and graduating with a BA in 1941. She worked on war-related research on the molecular structure of coals and was awarded a PhD from Cambridge in 1945. She then learned about x-ray crystallography at the Laboratoire Central des Services Chimique de l'Etat in Paris. Returning to England, she worked in the Biophysics Unit at King's College London and started to investigate DNA. Franklin took very clear x-ray diffraction images of DNA and showed that there were two forms--wet and dry--which produced very different pictures. The wet form she realized was probably helical in structure, with the phosphates on the outside of the ribose chains; by early 1953 she had concluded that both forms had two helices.

Maurice Hugh Frederick Wilkins was born in1916, in New Zealand to immigrant doctors from Ireland. His family returned to Dublin in 1923, but Irish politics forced them to move to Birmingham in the UK. In 1935, he enrolled at the University of Cambridge's St John's College, but graduated in 1938 with a lower-second-class degree, not good enough for graduate school at Cambridge. One of his teachers at Cambridge had become head of physics at the University of Birmingham, where Wilkins became a graduate student, gaining a Ph.D. in 1940. He moved with his doctoral supervisor to the University of St Andrews in Scotland and then to King's College, London, where he took up a career in biophysics, hoping to discover the molecular structure of genes. Following the work of Avery mentioned earlier, who had demonstrated that genes are DNA, he noticed under the microscope that fibers of DNA looked remarkably uniform and regular. If they were crystalline, he knew they would make a perfect target for X-ray diffraction. Wilkins drew DNA into microfibers. Gosling, his Ph. D student, found that under suitable conditions the DNA microfibers were crystalline. A physicist at King's, Alexander Stokes, looked at these diffraction patterns and concluded that DNA molecules were probably helix-shaped. In November 1950 Pauling had published a short letter announcing his discovery of helical protein molecules. Rosalind Franklin arrived at Kings in 1951 but the two did not get on and although in the same laboratory, they worked separately on the structure of DNA.

What happened next is a matter of controversy. It is clear that there was enmity between Franklin and Wilkins, and indeed between many of their superiors, and Wilkins need psychiatric help. In 1951, 23-year-old James Watson, a Chicago-born American, arrived at in Cambridge to work in the renowned Cavendish Laboratory. Watson had two degrees in zoology: a bachelor's degree from the University of Chicago and a doctorate from Indiana University, where he became interested in genetics. Watson went to Denmark for postdoctoral work, to continue studying viruses and to remedy his relative ignorance of chemistry. At a conference in the spring of 1951 at the Zoological Station at Naples, Watson heard Wilkins talk on the molecular structure of DNA and saw his recent X-ray crystallographic photographs of DNA. At the Cavendish, there were several significant X-ray crystallographic studies underway, under the leadership of Lawrence Bragg. Working under Max Perutz on the structure of hemoglobin was Francis Crick, who had a bachelor's degree in physics from University College London and he was recruited to work on DNA. Inspired by Pauling's success in working with molecular models, Watson and Crick rapidly put together several models of DNA and attempted to incorporate all the evidence they could gather. Franklin's excellent X-ray photographs, to which they had apparently gained access without

her permission, were significant in their theories. There were serious arguments and recriminations between the Cambridge and Kings groups, involving their laboratory directors. The four scientists announced the structure of DNA in separate articles in the same issue of *Nature*, in April 1953, one from Watson & Crick; one from Wilkins, Stokes & Wilson; and one from Franklin & Gosling.

In a probably apocryphal account, Watson and Crick announced to the world that they had discovered the 'secret of life' to fellow academics at the Eagle Pub in Cambridge. Arguments between the scientists continued, not helped at all by the publication much later, and long after Franklin's death, of a memoir by Watson in which he made scurrilous comments about her[233].

Franklin then moved to Birkbeck College and made important contributions to the X-ray crystallographic analysis of the structure of the tobacco mosaic virus. However, before she died from cancer, she had become friends with Francis Crick and had moved her laboratory to Cambridge, where she undertook work on the poliovirus. Watson's subsequent career eventually took him back to the USA to the Cold Spring Harbor Laboratory of Quantitative Biology where as. director from 1968. headed the National Center for Human Genome Research at the National Institutes of Health.

Crick, Watson, and Wilkins shared the 1962 Nobel Prize in Medicine for the discovery of DNA's structure and its replication mechanism. Rosalind Franklin had died in 1958. The rules of the Nobel Prizes are that no more than three scientists can be recognized by the same award, and they cannot be awarded posthumously. Thus, there were two, ostensibly valid, reasons why Franklin could have not been included in the 1962 prize, but this has, correctly, been a source of contention with respect to the recognition of the work of female scientists ever since.

2.3.3.3 Genes and Chromosomes

Having discussed the molecular structure of nucleic acids, it is now necessary to consider how they are incorporated into the human cell. Although there are many DNA-based molecular forms, which could be dealt with on individual levels, it is more informative to consider the structural features within a continuum, from DNA through to chromosomes, v*ia* genes. Put very simply, the sequence starts with DNA, which attaches to proteins called histones to form nucleosomes, which coil to form chromatids, which condense into chromosomes; a gene is a segment of the DNA that is used to produce one protein.

The essence of this discussion lies within the complexity of the structure and functions of DNA, and the need to protect its structure and enable a myriad of functions, all within the confines of human cells, and usually within the nuclei of those cells, which may be less than 10 μm in diameter. The DNA double helix is approximately 10 nm wide. The DNA molecule must be replicated when a cell is ready to divide, and the instructions that the molecule contains have to be used to create those molecules, especially proteins, that any specific cell needs for its functions. The DNA therefore has to be packaged in a very precise, specific ordered way to fit within the cell nucleus. A cell's complete complement of DNA is called its genome; the human genome is roughly 3 *billion* base pairs. The DNA is wrapped around proteins known as histones, the nature of the wrapping introducing several additional levels of complexity. DNA and the histones that it is wrapped around is called chromatin**. A**nimals and plants, have chromosomes that consist of several linear DNA molecules. These are thread-like structures located inside the nucleus of cells, each chromosome being made of protein and a single linear double-helix of DNA.

[233] Watson, J, The Double Helix: A Personal Account of the Discovery of the Structure of DNA, Atheneum Press, 1968.

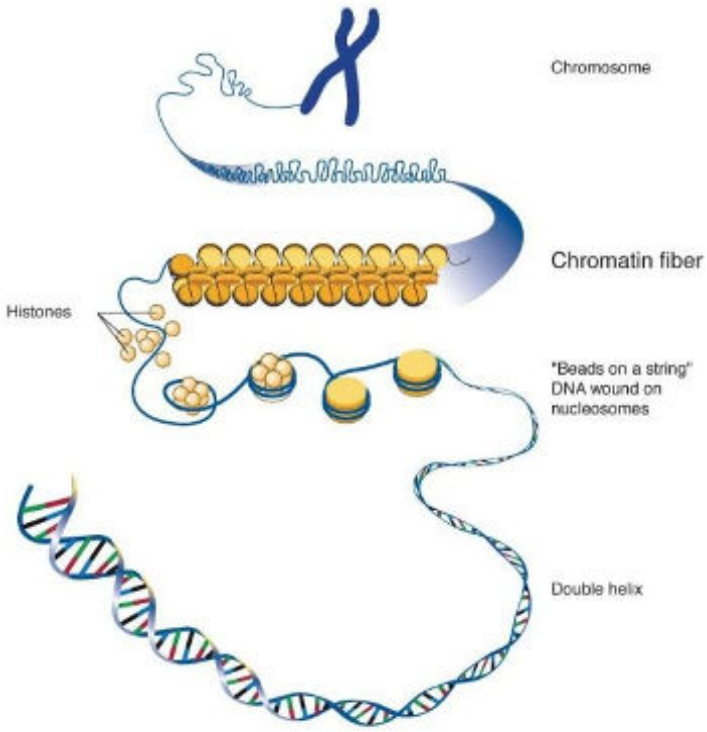

Figure 2.63. The hierarchy of DNA to chromosome structures.

Each species of eukaryote has a characteristic number of chromosomes in the nuclei of its cells. Chromosomes contain segments of DNA, which are the genes. Human body cells have 46 chromosomes. A somatic cell contains two matched sets of chromosomes, one from the male parent and one from the female parent. Eggs and sperm each contain one set of 23 chromosomes.

2.3.3.4 Characteristics and Properties of Genes; The Human Genome

A histone provides structural support for a chromosome; each chromosome contains a long molecule of DNA, which must fit into the cell nucleus, so the DNA wraps around complexes of histone proteins, giving the chromosome a more compact shape. The histones are positively-charged basic proteins that bind to negatively-charged phosphate molecules of DNA. There are five families of histones, one linker group, H1/H5, and four core groups, H2A, H2B, H3 and H4. Eight histone proteins can come together to make up a nucleosome around which the DNA can wind. Specifically, two each of the histones H2A, H2B, H3, and H4 come together to form a histone octamer, which binds and wraps approximately 1.7 turns of DNA, or about 146 base pairs. The addition of one H1 protein wraps another 20 base pairs, resulting in two full turns around the octamer. Thus, every chromosome contains hundreds of thousands of nucleosomes, and these nucleosomes are joined by the DNA that runs between them (an average of about 20 base pairs). This joining DNA is referred to as linker DNA. Each chromosome is thus a long chain of nucleosomes. Chromatin is a packaging material for DNA. It is a complex of nucleic acids and proteins and is the essential carrier of genetic information. The length of linker DNA can vary depending upon the gene activity and can range between 10 to 95 base pairs. There is a nucleosome after every 200 base pairs and its length was 10 nm.

As noted above, a gene is a defined segment of DNA. The principal functions of genes lie within the phenomenon of gene expression, which is the process whereby information that is stored in the DNA is converted into instructions for making proteins. This is tightly regulated, allowing genes to respond to changing environmental features, acting as both 'on' and 'off' switches that control when proteins are made, and the volumes of proteins that are produced. There are two stages, those of transcription and translation. In transcription, the DNA is copied to produce an RNA transcript, the messenger RNA, mRNA. This reaction is carried out by the enzyme RNA polymerase which uses available bases from the nucleus; the RNA molecule has only a single strand of bases, using the U base rather than T. In translation, the protein is made within the cell by ribosomes, the message being carried by transfer RNA, tRNA. The mRNA is read three units at a time, these being referred to as codons. Codons are made up of any triplet combination of the four nitrogenous bases A, G, C, U of nucleotides, that either encode information for the production of a specific amino acid or for the termination of protein syntheses. Of the 64 possible codon sequences, 61 specify the 20 amino acids of proteins and 3 are stop signals. When the mRNA sequence is read, each tRNA molecule delivers its amino acid to the ribosome and binds temporarily to the corresponding codon on the mRNA; adjacent amino acids join together to form a polypeptide.

2.3.3.4.1 Genetic Disorders

The reason why space has been devoted to genetics in this book is that a number of conditions which are, or could be, amenable to reconstructive procedures have their origins in genetic disorders and/or are inherited. As noted elsewhere, we can be as much concerned about reconstructing genes as about whole hearts or joints. Some of these genetic or inherited disorders will be discussed at appropriate place later, but a few of them are considered here in order to put them into some generic context.

Achondroplasia is a disorder of bone growth. It is caused by a mutation in the *FGFR3* gene that makes fibroblast growth factor receptor 3, which is involved in converting cartilage to bone. *It is* the only gene known to be associated with achondroplasia. All people who have only a single copy of the normal *FGFR3* gene and a single copy of the *FGFR3* gene mutation have achondroplasia. Most of those affected have average-size parents, and the mutation occurs in one parent's egg or sperm cell before conception. Others inherit the condition from a parent who has achondroplasia, in an autosomal dominant manner. Those who have achondroplasia have abnormal bone growth giving short stature with disproportionately short arms, legs and fingers, a large head with a prominent forehead and mid-face hypoplasia. The intelligence and life span in individuals with achondroplasia is usually normal. Genetic testing can identify mutations in 99 percent of individuals who have achondroplasia.

Autism Spectrum Disorders (ASDs) represents a broad group of developmental disorders characterized by impaired social interactions, problems with verbal and nonverbal communication, and repetitive behaviors or severely limited activities and interests. ASDs are the most commonly diagnosed childhood developmental disorder, affecting 1 in every 150 children in the USA. There is uncertainty over the causes of ASDs, although both genetic and environmental factors are likely to be involved. The majority are idiopathic, of unknown cause, with less than 15% having a chromosome abnormality with a single gene disorder. Some abnormalities in neurotransmitters indicate that autism may result from the disruption of normal brain development early in fetal development caused by defects in genes that control brain growth.

Cystic fibrosis is the most common, fatal genetic disease in the USA, where some 30,000 people are affected. The disease causes the body to produce thick mucus that accumulates in the lungs and blocks the pancreas, disrupting the movement of digestive enzymes to the intestine. It is caused by mutations in a single gene, the Cystic Fibrosis Transmembrane Regulator (CFTR) gene; more than 900 mutations of this single gene have been identified. However, more than 10 million people in the USA are symptom-less carriers of the defective gene and can pass it to their children. To develop the disease, a child must inherit

a defective gene from both parents, where there is a 25 percent chance that each child they conceive will have the condition, and a 50 percent chance that they will be a carrier. Cystic fibrosis has a variety of symptoms, including salty-tasting skin, a persistent cough and excessive appetite but poor weight gain. Gene therapy offers significant promise for life-saving treatment since it targets the cause rather than just treating symptoms.

Hemophilia is a disorder that affects the blood clotting process, resulting in excessive bleeding after injury and, in severe cases, spontaneous bleeding into the joints and muscles. Hemophilia occurs more commonly in males. There are two common types, hemophilia A in which there are low levels of the blood clotting factor eight (FVIII) and hemophilia B where there are low levels of factor nine (FIX), these two types arising from mutations in different genes. Genetic testing of the FVIII gene finds a disease-causing mutation in up to 98 percent of individuals who have hemophilia A, while that of the FIX gene finds disease-causing mutations in more than 99 percent of individuals who have hemophilia B. There is currently no cure for hemophilia. Hemophilia is inherited in an X-linked recessive pattern, where the mutation is located on the X chromosome. In males, one altered copy of the gene in each cell is sufficient to cause the condition; fathers cannot pass X-linked traits to their sons. With females, a mutation must be present in both copies of the gene to cause the hemophilia.

2.3.3.4.2 The Human Genome

The human genome represents the totality of knowledge about the approximately three billion base pairs of DNA that make up the entire set of chromosomes in humans. This includes the coding regions of DNA, which encode all the 20,000 - 25,000 human genes as well as the noncoding regions of DNA, which do not encode any genes. According to Goldman and Landweber[234] when the term 'genome' was initially introduced in 1920, it was intended to describe '*the haploid chromosome set which, together with the pertinent protoplasm, specifies the material foundation of the species'*. Back then, it was not known whether the genetic information was carried by DNA or by the protein component of chromosomes. Today, with that extra knowledge, the concept has been modified to '*all the information needed to build and maintain an organism, through a complete set of DNA, including all of the genes*. The human genome is neither uniform nor static. Excepting identical (monozygous) twins, no two humans share exactly the same genomic sequence. Changes arise with considerable frequency. Some of these changes are neutral or even advantageous and eventually become commonplace in the population. Others may result in reduced survival or fertility. The genome is a record of the performance of the previous generations.

This book is not the place to describe the technicalities of the Human Genome Project in any more detail; an excellent introduction to this was published by Hood and Rowen a few years ago[235].

2.3.3.4.3 Spirituality,faiths and the Human Genome Project

The Hood and Rowen paper cited above made it very clear that there was considerable resistance to the Human Genome Project when it was initially discussed in the USA in the mid 1980s, mostly because scientists did not think it would produce any benefit. There is, of course, much more of a debate about the genome, as explained by Hamer in the provocatively titled 2004 book 'The God Gene"[236]. The book does not refer to genes for being a god, but about those genes that underlie spiritual beliefs and experiences. Spirituality, he contends is not controlled by the product of a single gene, but of many, each making their own contribution to the phenotype, in parallel with environmental influences. Genetic factors may have

[234] Goldman AD and Landweber LF, What is a genome?, *PLoS Genetics*, 2016;e1006181. doi:10.1371/journal.ogen.1006181.
[235] Hood L and Rowen L, The Human genome project: big science transforms biology and medicine, *Genome Medicine,* 2013;5:79. doi:10.1186/gm483.
[236] Hamer D, *The God Gene*: How faith is hardwired into our genes, 2004, Doubleday, ISBN 0-38550-058-0.

been favored during evolution since spirituality appears to have some positive effect on the ability to reproduce.

At least in this area of spirituality, there is some data to work with[237]. Information mainly relates to single nucleotide polymorphisms, specifically single gene mutations in neurotransmitters, their transporters or related hormones. Serotonin is one such molecule, which has been implicated in personality; the serotonin receptor binding potential is reduced in the brains of depressed individuals. 5-HT2A receptor gene polymorphism rs6311modulates HTR2A promoter activity, such that activity is greater in the presence of the A allele relative to the G allele. With dopamine, several abnormalities have been found in dopamine receptor genes, especially DRD2 gene. In the context of the neurobiology of spirituality, there are similarities in the functional activity in spiritual and religious experiences and the dopaminergic systems. It is interesting to note that spirituality and its expression as religiousness may be a phenotype of receptor genes, such as DRD2, in people at low risk of depression, but a high familial risk of depression, i.e., environmentally-related, may suppress the phenotypic expression of these genes that are normally associated with spirituality.

The broader ethical issues concerning the Human Genome project have been covered elsewhere. Soon after the initiation of the project. The United Nations, and specifically the UN Educational, Scientific and Cultural Organization (UNESCO) adopted a Declaration on the Human Genome in relation to human rights[238]. The preamble to this Declaration states that" research *on the human genome and the resulting applications open up vast prospects for progress in improving the health of individuals and of humankind as a whole, but such research should fully respect human dignity, freedom and human rights, as well as the prohibition of all forms of discrimination based on genetic characteristics*". Those sentiments are enshrined in many of the Articles of the Declaration, including 2(b)" that dignity makes it imperative not to reduce individuals to their genetic characteristics and to respect their uniqueness and diversity" and 5(e) that research which does not have a direct health benefit may only be undertaken by way of exception, with the utmost restraint, exposing the person only to a minimal risk".

2.3.3.5 Characteristics and Properties of Chromosomes; The Biology of Inheritance

2.3.3.5.1 Structure of human chromosomes

A chromosome is made of a very long strand of DNA and contains hundreds to thousands of genes; chromosomes also contain other chemicals that influence gene function. The genes on each chromosome are arranged in a particular sequence, and each gene has a specific location on the chromosome, known as its locus). Except for certain cells (for example, sperm and egg cells), the nucleus of every normal human cell contains 23 pairs of chromosomes, giving a total of 46. Each pair usually consists of one chromosome from the mother and one from the father. There are 22 pairs of nonsex (autosomal) chromosomes. Paired nonsex chromosomes are essentially identical in size, shape, position and number of genes they contain. The 23rd pair are the sex chromosomes (X and Y), which determine whether a fetus becomes male or female. Males have one X and one Y chromosome, the X coming from the mother and the Y from his father. Females have two X chromosomes, one each from the mother and father.

The smaller Y chromosome carries the genes that determine male sex. The X chromosome contains many more genes, with several functions besides determining sex; there is no counterpart on the Y

[237] Anderson MR, Miller L, Wickramaratne P, *et al*, Genetic correlated of spirituality / religion and depression: A stusy in offspring and grandchildren at high and low familial risk for depression, *Spiritual Clinical Practice,* 2017;4(10:43-63. doi:10.1037/scp0000125.

[238] United Nations Educational, Scientific and Cultural Organization, *Universal Declaration on the Human Genome and Human Rights*, Adopted in Geneva, 11th November 1997.

chromosome. Genes on the X chromosome are referred to as sex-linked, or X-linked, genes. Normally, in the nonsex chromosomes, the genes on both of the pairs of chromosomes are capable of being fully expressed. However, in females, most of the genes on one of the two X chromosomes are turned off early in the life of the fetus through a X inactivation. In some cells, the X from the father becomes inactive, and in other cells, the X from the mother becomes inactive. Thus, one cell may have a gene from the mother and another cell has the gene from the father. Because of X inactivation, the absence of one X chromosome usually results in relatively minor abnormalities. Thus, missing an X chromosome is far less harmful than missing a nonsex chromosome. A person may have an abnormal number of chromosomes or have abnormal areas on one or more chromosomes. Abnormal numbers of nonsex chromosomes usually result in severe abnormalities; having an extra non-sex chromosome may be fatal to a fetus or lead to abnormalities such as Down syndrome, which normally results from a person having three copies of chromosome 21.

The general structure of a chromosome is seen below

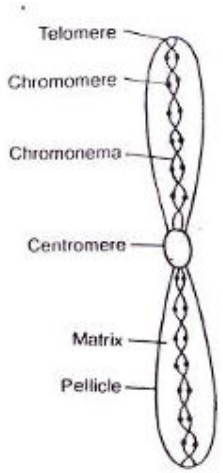

Figure 2.64. General structure of a chromosome.

The primary constricted portion of a chromosome is known as centromere, which is the specific part where spindle fibers are attached. Its position is constant for a particular chromosome. The characteristics of the centromere are different from those of the rest of chromosome. During cell division, the centromere is functional while the remainder is genetically inactive. In mitosis, metaphasic chromosomes consist of two chromatids and four granules, known as centromeric chromomeres, are seen within the centromere. During anaphase, the two chromatids separate, each chromatid showing two granules. Simultaneously, duplication of the centromere occurs during mitosis and meiosis.

A membrane known as pellicle, surrounds each chromosome, and a gelatinous substance, the matrix, is present inside the pellicle. During early prophase, a chromosome appears to consist of filamentous long continuous structures called chromomeres. The number and position of each chromomere is relatively constant in a chromosome These chromomeres are responsible to carry the genes during inheritance.

The chromosomes of eukaryotes are linear. This implies that they have ends, which pose a problem for DNA replication. The DNA at the very end of the chromosome cannot be fully protected, resulting in a slow, gradual shortening of the chromosome. Part of the DNA at the end of the chromosome goes uncopied in each round of replication, leaving a single-stranded overhang. Over multiple rounds of cell division, the chromosome will get shorter and shorter as this process repeats. To prevent the loss of genes

as chromosome ends wear down, the tips of eukaryotic chromosomes have specialized DNA “caps”, which are called telomeres. These consist of hundreds of repeats of the same short DNA sequence. In humans, the single-stranded overhangs in the caps bind to complementary repeats in the nearby double-stranded DNA, causing the telomere ends to form protective loops. Also, proteins associated with the telomere ends help protect them and prevent them from triggering DNA repair pathways. Some cells have the ability to reverse telomere shortening by expressing telomerase, an enzyme that extends the telomeres of chromosomes. The phenomenon of chromosome shortening and the role of telomeres has become an important issue in regenerative medicine technologies, as noted in a later section.

Human chromosomes have different shapes, sizes and configurations. Depending on the position of the centromere, and the lengths of the arms, the shapes are classified as

- Acrocentric, being I-shaped, with unequal arms, and the position of centromere is sub-terminal.
- Sub-metacentric (Heterobrachial),‘L’ or J-shaped. The centromere is not in the centre of the chromosome and arms are unequal.
- Metacentric (Isobrachial): V shaped. Centromere is present in the centre of chromosome and arms are equal in length.
- Telocentric, I-shaped, single armed, with a terminal centromere.
- Dicentric or Polycentric, with two or more centromeres.
- Acentric, with no centromere in the chromosome.

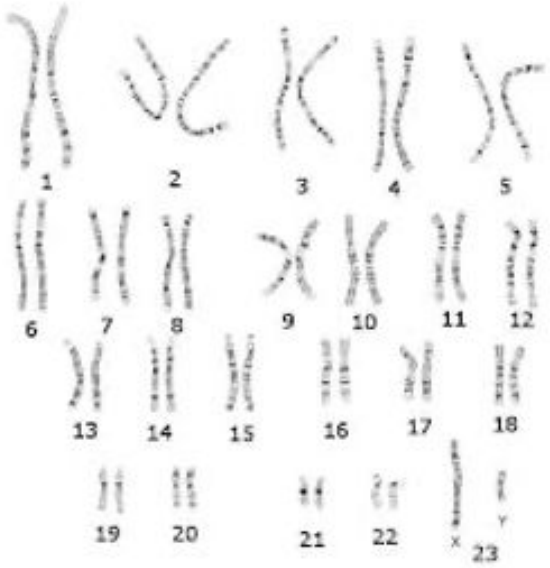

Figure 2.65. Shape of human chromosomes.

The actual location of particular genes on a chromosome can be described in terms of cytogenetic or molecular information, the former being most widely used although there is more precision with the latter. The cytogenetic location is defined by combinations of numbers and letters, typically a number, a letter and then another number. Thus, the first number is the chromosome number. The letter represents the arm of the chromosome, by convention the shorter arm being called 'p' and the longer arm 'q'. The final number represents the position of the gene on that arm' represented by bands and sub-bands. For example, the cytogenetic map location of the Cystic Fibrosis Transmembrane Conductance Regulator gene is 7q31.2, which indicates it is on chromosome 7, q arm, band 3, sub-band 1, and sub-sub-band 2.

2.3.3.5.2 Chromosome instability

Chromosomal instability syndromes are a group of inherited disorders associated with chromosomal instability and breakage, either spontaneously or in response to DNA damaging agents. The majority of these syndromes are significant because they have associations with variable degrees of immunodeficiency, infectious disease, and the risk of developing certain types of malignancies. The most important disorders are as follows;

- Ataxia-telangiectasia is an autosomal recessive disorder that primarily presents with cerebellar ataxia, which results from a mutation in ATM (ataxia telangiectasia mutated) gene, which leads to a loss of ATM protein. This protein in normal conditions recognize DNA damage and activates DNA repair mechanisms to reduce genetic damage.
- Bloom syndrome is an autosomal recessive disease caused by a lack of BLM helicase enzyme, the result of a mutation in the BLM gene. Symptoms include disproportionately short stature, microcephaly, immunodeficiency, sinopulmonary infections, decreased intellectual ability and facial anomalies.
- Fanconi anemia is a DNA repair disorder where cells cannot repair DNA damage caused by interstrand cross-links, which eventually leads to chromosomal instability, particularly upon exposure to cytotoxic therapies and a general predisposition to certain cancers.
- Nijmegen breakage syndrome is an autosomal recessive chromosome instability syndrome associated with immunodeficiency. This is a result of mutations in the nibrin (NBN) gene on 8q21. The protein product is involved in DNA double-strand breaks repair, base excision repair, meiotic recombination, and telomere maintenance. Progressive symptoms include microcephaly, facial deformities, intrauterine growth retardation, intellectual disability, a predisposition to lymphoid malignancies, primary ovarian insufficiency, and radiosensitivity.

For reference, genetic disorders may be dominant or recessive. Dominant diseases can be caused by only one copy of a gene having a DNA mutation. If one parent has the disease, each child has a 50% chance of inheriting the mutated gene. With recessive diseases, both copies of a gene must have a DNA mutation. If both parents have one copy of the mutated gene, each child has a 25% chance of having the disease, even though neither parent has the disease. In such cases, each parent is called a carrier of the disease.

Although many of these inherited conditions are quite rare, it is easy to see how the resulting deformities should be attractive to the technologies of reconstruction, including gene therapy and tissue regeneration or replacement.

2.4 CELLULAR CHARACTERISTICS

There are several hundred different types of cell in the human body; probably not a surprising statistic since we each contain several trillion cells and since our bodies have so many different functions that we demand of these cells. Their classification is not a trivial matter, partly because some of them have very similar features and since their phenotype (characteristics defined by morphology and function) can

change in a subtle manner. I just provide enough detail here to allow some understanding of these cells as relevant to reconstructive and regenerative procedures. It is worth remembering that some cell types are persistent, undergoing constant renewal in healthy individuals since their function is essential to sustained health, and indeed life. Other cells may be produced within certain compartment of the body upon demand, for example during infectious or traumatic periods.

A recent (2022) book on the characteristics of cells and their importance in medicine, provides a very balanced account on this subject, being detailed yet written in largely layman's language[239]. Interestingly in the context of the present book, Mukherjee considers that progress in medicine today has been underpinned by four developments in cell biology, namely the use of drugs to alter the properties of cells, the transfer of cells between bodies, the use of cells to synthesize therapeutic substances, and the genetic modification of cells. These developments form integral parts of the later chapter on enabling technologies. Mukherjee believes that the applications of these cell-based systems is resulting in 'new humans'; I will refer to this concept a few times myself.

The simplest, and perhaps most logical, way to classify cells involves those parts of the embryo from which they were initially derived. There are three such parts, the endoderm, ectoderm and mesoderm. There is some overlap and the appropriation to any one embryo layer means that they are primarily, if not exclusively derived there. I list here the main groups of cells within these three groupings and relevant examples in each group; it will evident that this is not an exhaustive list. Certain generically relevant types of cell, including cancer cells and stem cells will be discussed at the end of the section.

2.4.1 Fundamental Structure and Function

Although we are exclusively considering humans, it is helpful to briefly put the structure and function of cells of the human body into a broader biological context.

2.4.1.1 Membranes

The cell can be regarded as the basic unit of life and is separated from everything else by a flexible film known as the cell membrane (also called the plasma membrane). Equally importantly, within most cells, the chemical reactions that take place do so in confined, well-defined areas, which are also separated by membranes. The existence and characteristics of these membranes determine what functions the cell can perform. The cell membrane consists of a lipid bilayer that is semipermeable and is able to regulate the transport of substances entering and exiting the cell. It provides structural support for the cell, and a fixed environment inside the cell. It will have proteins on it that interact with other cells; these may be glycoproteins, which combine sugar and protein moieties, or lipid proteins, combining fat protein moieties.

Some types of cells do not contain any membrane-bound structures. These are prokaryotes, typically being unicellular organisms such as bacteria. Although these do not have internal membranes, they do have distinct cellular regions. They do not have a nucleus but do have central region, the nucleoid, which contains DNA. They have ribosomes that are responsible for protein synthesis, and may have features on their cell membrane, such as fimbriae, that facilitate attachment to surfaces. This book only discusses prokaryotes in the context of bacterial infections.

[239] Mukherjee S, "*The Song of the Cell; An Exploration of Medicine and the New Human",* Scribner, New York, 2022.

2.4.1.2 Internal Structures in Eukaryotic Cells

Virtually all other types of cell, and specifically those found in animals, plants, fungi and algae, have internal membrane bound structures that are able to carry out specific functions; these cells are known as eukaryotes, derived from the Greek 'eu' which means well or good, and karyo, meaning nut or kernel. They are typically within the size range of 10 to 100 microns. The primary components of eukaryotic cells are the nucleus, which stores genetic information, the nucleolus, found inside the nucleus and responsible for RNA synthesis, the cytoskeleton which provides structure, ribosomes that synthesize proteins, the mitochondria that are responsible for energy production, the endoplasmic reticulum that is responsible for protein transportation, the cytoplasm which is the region between the nucleus and the cell membrane and which contains the gel-like cytosol, and various vesicles and vacuoles.

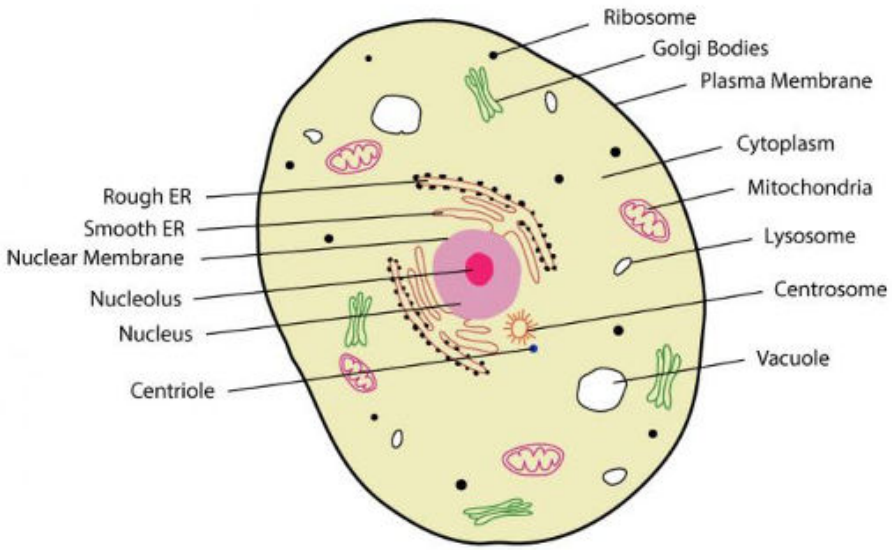

Figure 2.66. General structure of animal cell.

The nucleus is separated from the rest of the cell by a double layer, the nuclear membrane. The nucleus controls and regulates the activities of the cell, such as growth and metabolism. Since the nucleus houses the host's genetic code, which determines the amino acid sequence of the proteins, it primarily serves as the information center of the cell. Information contained in the DNA is transcribed into messenger ribonucleic acid (mRNA) molecules, each of which encodes the information for one protein. The mRNA molecules are transported through the nuclear envelope into the cytoplasm for translation, serving as templates for the synthesis of specific proteins. The nucleolus is contained within the nucleus and produced ribosomal RNA. The nuclear components are suspended is the nucleoplasm, a gel-like matrix. While a cell normally contains only one nucleus, sometimes the nucleus divides, producing a multinucleate cell, one of which type plays a very important part in the inflammatory responses to some biomaterials. Also, some cells, especially the red blood cell, lose their nuclei upon maturation.

2.4.1.3 Energy Conversion

Energy conversion is a principal function of human cells. Cells derive energy from food-derived molecules such as sugars, fats and proteins; as these molecules are broken down chemically within the cell, electron acceptor molecules capture some of the energy released during the reactions, converting it into small energy-rich molecules such as ATP, adenosine triphosphate, and NADH, nicotinamide adenine dinucleotide. The eukaryotic energy pathway then has three phases, glycolysis, the citric acid cycle and oxidative phosphorylation. Glycolysis is the breakdown of sugar molecules which are converted into pyruvate molecules, which enter mitochondria and are converted into acetyl CoA. In oxidative phosphorylation, electrons are transferred from NADH through membrane protein complexes, generating a proton gradient across the mitochondrial membrane, providing the energy that becomes available to the cell.

It is not surprising that the complexity of the structure and function of the many different types of cell in the human body has led to a variety of diseases and conditions in which the principal cause is the absence or dysfunction of one or more cell type. At its most elementary level, reconstruction of the body has to address the possibility of replacing deficient with functioning cells. Such therapies are collectively referred to as cell therapies, and these are considered at appropriate places in this book. It is necessary to describe the various types of cells of the body to place these possibilities in perspective. There are several ways in which human cells could be classified; I choose to use the germ layer of origin as the primary feature, i.e., those derived from endoderm, mesoderm and ectoderm, and then describe some of the exemplar cells or families of cells within these three groups. This is not a precise schema, since some cells are derived from two germ layers, but it is convenient in the context of the purpose of this section.

2.4.1.4 Cell Division

During the early days of cell biology, in the mid-nineteenth century, many details of cell structure and function were being unraveled but the answer to the question, where do cells come from, remained elusive. The very simple answer to this fundamental question, that all cells are derived by the division of precursor cells, arose during the aftermath of the series of revolutions in Europe around 1848. The apparent discoverer of this fact, Rudolf Virchow, has been credited with the concept of "*Omnis cellula e cellula*", or "*from cells come cells*", delivering this in Wurzburg, Germany in 1858. As noted by Wright and Poulsom (and several others), there is some doubt as to whether this was the case, and Virchow appears to have 'borrowed' the concept and phrase from earlier scientists such as Theodore Schwann, Robert Remak and Francois- Vincent Raspail. Nevertheless, the credit largely vests with Virchow and with it the disciple of cellular pathology and the understanding of cell division.

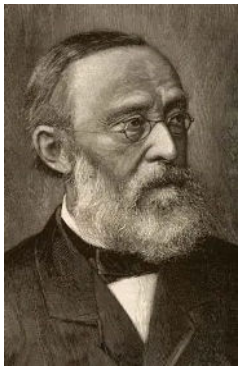

Figure 2.67 Rudolf Virchow, 'Father' of cellular pathology.

Survival of eukaryotes depends on a balanced distribution of the many types of constituent cells, which is achieved by highly regulated processes of cell proliferation. The growth and division of different cell populations are regulated in different ways; in adults, cell division is primarily involved in tissue renewal rather than growth, many types of cells undergoing continuous replacement. Before a cell can divide, the genetic information encoded in its DNA must be accurately and completely duplicated. In eukaryotes the processes of DNA replication and cell division occur at different times of the cell division cycle. During cell division, DNA condenses to form short, tightly coiled, chromosomes, which then split longitudinally, forming two identical chromatids. Each pair of chromatids is divided between the two daughter cells during mitosis, the division of the nucleus. A major feature of mitosis is the attachment of the chromatids to opposite poles of the mitotic spindle (the collective term for the fibers that form during mitosis which are responsible for moving and segregating the chromosomes during nuclear division), which ensures that each of the daughter cells will receive a complete set of chromosomes. There are five phases of mitosis. First, in prophase, the mitotic spindle forms and the chromosomes condense. Next, in prometaphase the nuclear envelope breaks down and the chromosomes attach to the mitotic spindle. In metaphase the condensed chromosomes align in a plane across the equator of the mitotic spindle, and in anaphase the separated chromatids move towards opposite spindle poles. In the final stage, telophase, a new nuclear envelope forms around each set of unraveling chromatids.

A specialized division of chromosomes, meiosis, occurs during the formation of the reproductive cells, (gametes) of sexually reproducing organisms; this is not considered here.

2.4.1.5 Spiritual Aspects of Cells

Since the discovery of the cell is relatively recent, there are no commentaries on spirituality aspects in historical texts. The discovery itself was based on the work of British scientist Robert Hooke, who developed microscopes to study material microstructure, and observed very small pores in cork, which he called 'cells'[240]. His observations were noted by the Dutch scientist, Antonie van Leeuwenhoek, who improved the microscope and saw moving biological cells in many environments[241]. There have, of course, been several attempts to implicate spiritual factors in the treatment of cell-based diseases, but collectively without conviction or proof. For some time, cancer was the target of spiritual healing in this context, but this was clearly shown to be misguided in the paper by Zachariae *et al* who concluded that "*reported beneficial effects of spiritual healing on the well-being of cancer patients seems more likely to be mediated by psychosocial and psychophysiological effects on the healer-patient relationship*"[242].

2.4.2 Cells Derived from Endoderm (Inner Layer of Embryo)

The endoderm is one of the earliest parts to form in the embryo. It is the progenitor tissue that gives rise to much of the internal organ systems, including the respiratory and gastrointestinal tracts, and their associated organs such as the thyroid, liver, pancreas, prostate, and bladder. Endodermal tissues are

[240] Robert Hooke*, Micrographia: Some physiological descriptions of minute bodies made by magnifying glasses with observations and inquiries thereon*. The Royal Society, London, 1665, Published Online by The Royal Society, 2020.
[241] Lane N, The unseen world" reflections on Leeuwenhoek (1677) 'Concerning little animals'. *Philosophical Transactions of the Royal Society B,* 2015;370 (Issue 1666). doi:/10.1098/rstb.2014.0344.
[242] Zachariae R, Højgaard L, Zachariae C, *et al,* The effect of spiritual healing on in vitro tumor cell proliferation and viability – an experimental study. *British Journal of Cancer*, 2005;93(5):538-43. doi:10.1038/sj.bjc.6602749.

necessary for many homeostatic processes, including absorption of nutrients, gas exchange, detoxification and glucose homeostasis.

2.4.2.1 Hormone-secreting cells

2.4.2.1.1 Enteroendocrine and other cells of the gastrointestinal tract[243]

Enteroendocrine cells are found within the gastrointestinal tract, stomach, and pancreas. They produce and release hormones either into the bloodstream to generate systemic effects or locally. Within the intestinal epithelium they are the most abundant form of endocrine cell in the body, even though they constitute only 1% of that epithelium.

There are five gastrointestinal hormones secreted by these cells: gastrin, cholecystokinin (CCK), secretin, glucose-dependent insulinotropic peptide (GIP) and motilin. There are sub-types of the enteroendocrine family of cells with responsibility for the secretion of these hormones. Gastrin is secreted by G cells, which are primarily involved in the upper part of the tract, especially the stomach, the gastrin being released in response to vagal and gastrin-releasing peptide. Gastrin acts through two mechanisms that increase the secretion of gastric acid into the stomach, the first involving gastrin binding to CCK-2 receptors on parietal cells and the second involving the release of histamine in response to the gastrin. I - cells secrete CCK and are located in the duodenum and jejunum and modulate bile secretion and exocrine pancreas secretion and K cells secrete GIP which also promotes triglyceride storage.

The pH of stomach fluid in humans is normally between 1.5 and 3.5. This is lower than for most mammals and reflects the evolution trends as man became omnivorous and needed to eat foods which were difficult to digest, and often infected by bacteria[244]. It is not surprising that such a degree of acidity has been problematic, and physicians have attempted to address these issues for many centuries[245]. Paracelsus recognized the presence of acidic fluid in the stomach over 500 years ago, but it was not until 1823 that Prout identified hydrochloric acid as the main constituent. Additional information about the role of pepsin and of the vagus nerve in the control of gastric acid secretion and its physiological effects seemed to answer many of the questions about gastric ulcers, but it was the discovery that Heliobacter pylori was the real causative factor by Marshall and Warren that changed the management of this widespread condition.

It was either perverse or insightful that the Irish author James Joyce did not shirk from addressing human illness, especially of the GI tract[246], and himself died of a perforated duodenal ulcer, in 1941 in Zurich. Perhaps his major publication, *Ulysses,* made him very famous, partly because of his style and mainly because it was widely censored. He used Homer's *Odysseus* as a template, in that "*each episode deals with a particular art or science which represented a special human organ, indeed has its particular color, technique, and takes place at a particular time*." All of the action of the novel takes place in Dublin on a single day in June 1904. The three central characters, Stephen Dedalus, Leopold Bloom, and his wife, Molly, are modern counterparts of Telemachus, Ulysses and Penelope, and using a variant of the technique of interior monologue, known as the stream-of-consciousness, Joyce exposes the innermost feelings of the characters as they live during the day. Of importance here is the 'Lestrygonians' episode, which makes reference to food and the 'peristaltic prose' as it passes though the GI tract. Bloom walks

[243] Engelstoft MS, Egerod KL, Lund ML, *et al,* Enteroendocrine cell types revisited, *Current Opinion in Pharmacology*, 2013;13:912-21. doi:10.1016/j.coph.2013.09.018.
[244] Fujimori S, Gastric acid level of humans must decrease in the future, *World Journal of Gastroenterology*, 2020;26(43):6706-9. doi:10.3748/wjg.v26.i43.6709.
[245] Herszenyi L, Bakucz T, Barabas L, *et al,* Pharmacological approaches to gastric acid suppression" Past, present and future, *Digestive Diseases,* 2020;38:104-11. doi:10.1159/000505204.
[246] Shanahan F and Quigley EMM, James Joyce and gastroenterology, *Clinical Medicine*, 2008;8(6):632-5.

through Dublin during the day and, in this episode, his own odyssey is represented by stops and starts, where he was 'eaten and spewed".

2.4.2.1.2. Cells of the Islets of Langerhans[247]

The islets of Langerhans are islands of endocrine cells, of mixed phenotype, that are scattered throughout the parenchyma of the pancreas; their number varies from 3 to 15 million. In humans, about 60% of these cells are insulin-producing ß-cells, 30% are glucagon-releasing α-cells, and 10% are somatostatin-producing δ-cells or pancreatic polypeptide-producing γ-cells, or ghrelin-producing ε-cells. The ß-cells contain insulin in granules as a complex with zinc, which is released in response to high glucose levels and following stimulation by neurotransmitters. Glucagon is the counter hormone to insulin; this counter-regulatory mechanism prevents hypoglycemia, which is impaired in diabetes. It is noteworthy that there is considerable heterogeneity in the structure and performance of ß-cells, as well as a degree of plasticity, such that non- ß-cells can be converted to ß-cells under some circumstances.

2.4.2.1.3. Cells of the thyroid and parathyroid glands

The thyroid gland is an endocrine gland located in the lower part of the neck. It consists of two lobes, the right and the left, joined together by an intermediate structure, the isthmus. It has a fibromuscular band, (levator glandulae thyroideae) running from the body of the hyoid to the isthmus. It grows larger in females during the period of menstruation and pregnancy. Two capsules completely cover the thyroid gland. The true capsule is made up of fibro-elastic connective tissue. The false capsule comprises the pre-tracheal layer of the deep cervical fascia. consisting of deep capillary plexus deep to the true capsule. The thyroid lobules consist of many units, the thyroid follicles, which are spherical, with walls of cuboidal follicular cells, the follicular cells, which secrete thyroid hormone. There are two forms of this hormone, predominantly thyroxine, which is tetraiodothyronine (T4), with a small quantity of triiodothyronine (T3). These hormones help in regulating the basal metabolic rate. In addition, parafollicular cells are located between the thyroid follicles, which are derived from neural crest cells. These secrete the polypeptide hormone calcitonin, which helps regulate calcium and phosphate deposition in skeletal and other tissues. Together, the thyroid hormones assist in the overall growth, development, and differentiation of all the body cells, regulating the basal metabolic rate and calcium metabolism They stimulate somatic and psychic growth and heart rate and contraction, with influence over carbohydrate, fat, protein, and vitamin metabolism.

Several important clinical conditions arise from thyroid gland dysfunction. Goiter is a condition where the gland shows an abnormal enlargement. Goiters are broadly classified into uni-nodular, multinodular, and diffuse types. Each further includes many different types of goiters. Colloid nodular goiter is the commonest non-neoplastic lesion, where the thyroid follicles are filled with an abundant amount of colloid in their lumens and lined by squamous follicular cells. Hyperthyroidism, or thyrotoxicosis is a hypermetabolic state, resulting in increased T3 and T4 levels, where symptoms included palpitations and tachycardia. Graves' disease is a combination of thyrotoxicosis, exophthalmos, and dermopathy, especially seen in women in the age group of 20 to 40 years, giving prolonged and violent palpitations. Hypothyroidism develops from functional and structural derangement and consequent decreased thyroid hormone production; it manifests as cretinism in infants and myxoedema in adults, where patients present as short-statured, with coarse facial features, and mental retardation. Thyroid carcinomas arise either from the follicular epithelium or parafollicular C-cells. They can manifest in the form of papillary carcinoma, follicular carcinoma, anaplastic carcinoma, and medullary carcinoma. Parathyroid glands are usually located in close association with the thyroid gland; typically, there are normally four individual parathyroids, two superior glands located near the posterolateral aspect of the superior pole of the thyroid and two inferior glands located near the inferior poles of the thyroid glands, within 1-2 cm of the insertion

[247] Xavier CDS, The cells of the Islets of Langerhans, *Journal of Clinical Medicine*, 2018;7:54. doi:10.3390/jcm7030054.

of the inferior thyroid artery into the inferior pole of the thyroid. They have two distinct types of cells: the chief cells and the oxyphil cells. The chief cells are responsible for the secretion of parathyroid hormone (PTH) but the purpose of the oxyphil cells is not entirely understood.

The parathyroid glands help regulate blood calcium levels; the gland releases PTH in response to low calcium levels, this initially being produced as a polypeptide hormone. In the kidney, PTH promotes calcium reabsorption and excretion of phosphate. In the intestines, activated vitamin D promotes the absorption of calcium following formation of the calcium-binding protein in the epithelial cells. In bone, PTH affects both osteoblastic and osteoclastic cells. When PTH binds to the cellular receptors, it allows the pumping of calcium from the osteocytic membrane, which allows for the rise of calcium to occur within minutes. A slower phase takes several days to precipitate an increase in blood serum calcium, occurring via osteoblasts. A hyperfunctioning parathyroid gland often requires surgical intervention. When the parathyroid over-secretes PTH, there are risks associated with elevated serum calcium. Primary hyperparathyroidism is due to direct gland alterations, most commonly due to a parathyroid gland adenoma. Secondary hyperparathyroidism is a physiological increase of PTH due to reduced calcium levels in the blood. The most common cause is chronic kidney disease but can also be due to vitamin D deficiency or malnutrition. Hypoparathyroidism is due to reduced activity of the gland; a primary condition involves a gland failure which results in a decrease in PTH secretion when patients often need calcium supplementation, while a secondary condition occurs following surgical removal or injury of the parathyroids, for example after extensive neck surgery or radiation therapy for neck malignancies.

2.4.2.2 Exocrine Secretory Epithelial Cells

Epithelial cells derive from all three embryonic layers. It is those cells that line the airways and much of the digestive system that originate in the endoderm.

As noted elsewhere in this section, epithelia are continuous sheets of cells that cover surfaces; there are two major types of epithelia, the covering and glandular epithelia. The latter are organized collections of secretory cells; they may be endocrine, which are glands the secretions of which are released directly into the blood stream, or exocrine, which are glands that have ducts where secretions are passed through the ducts and onto their respective epithelial surfaces.

The airway epithelium is essential for maintenance of the conduit for air to and from the alveoli, defending the lung against pathogens and inhaled particulates through the activity of secretory and ciliated cells[248]. In the large airways, the epithelium is pseudostratified, becoming cuboidal and columnar in smaller airways. There is turnover of the epithelium every 30 to 50 days in humans. It does, however, operate in a very aggressive environment, being exposed to a multitude of exogenous chemicals and agents, and it becomes deranged in several major pulmonary disorders, including chronic obstructive pulmonary disease (COPD), asthma and bronchogenic carcinoma. In the context of reconstructive therapies, it is interesting to speculate, as proposed by Weiss[249], that replacement of parts of this epithelial layer through yet to be perfected techniques, could be of benefit in this area of un-met clinical need.

The intestinal epithelium has many similarities to that of the airway, but with a significant difference, that of controlling the symbiotic ecological community in the gut[250]. The intestinal epithelial cells combine

[248] Crystal RG, Randell SH, Engelhardt JF, *et al*, Airway epithelial cells, *Proceedings of the American Thoracic Society,* 2008;5:772-777. doi:10.1513/pats.200805-041HR.
[249] Weiss DJ, Cell-based therapy for chronic obstructive pulmonary disease: Rebuilding the lung. *Annals of the American Thoracic Society*, 2018;15 (Suppl4): S253-S259. doi:10.1513/AnnalsATS.201808-534MG.
[250] Okumura R, Takeda K, Roles of intestinal epithelial cells in the maintenance of gut homeostasis, *Experimental and Molecular Medicine*, 2017;49:e338. doi:10.1038/emm.2017.20.

barrier with secretory functions to exert this control. So-called 'segregation' is the separation of the gut microflora from host immune cells, while 'mediation' involves delivery of signals between them, with secretion of cytokines and chemokines, and of specific antimicrobial molecules to regulate the over-growth of pathogenic opportunistic microbes. This balance can also be deranged, as seen with serious, life-destabilizing diseases of inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease. As with airway diseases these could be the target of cell and gene therapies in the future.

2.4.3 Cells Derived from Mesoderm[251] (Middle Layer of Embryo)

2.4.3.1 Bone and Cartilage Derived from the Paraxial Mesoderm[252]

There are three types of cell that are the origins of bone cells. The axial skeleton is derived from the paraxial mesoderm, while the appendicular skeleton originates in the lateral plate mesoderm. The neural crest actually arises from the ectoderm and is the origin of the craniofacial skeleton.

There are two major modes of bone formation, and both involve the transformation of a preexisting mesenchymal tissue into bone tissue. Intramembranous ossification is the direct conversion of mesenchymal tissue into bone. In endochondral ossification**,** mesenchymal cells first differentiate into cartilage, which is later replaced by bone; this is the dominant mode in much of the body. Endochondral ossification takes place in five stages. First, mesenchymal cells are committed to become cartilage cells, through the action of paracrine factors that induce mesodermal cells to express two transcription factors, Pax1 and Scleraxis. The committed mesenchyme cells then condense into compact nodules and differentiate into chondrocytes, which are the cartilage cells. In humans, the SOX9 gene is expressed in the pre-cartilaginous condensations. Mutations of this gene cause campomelic dysplasia, a usually fatal condition that results in deformities of most of the bones of the body. During the third phase, the chondrocytes proliferate rapidly; as they divide, they secrete a cartilage-specific extracellular matrix. They then stop dividing and increase their volume dramatically, altering the matrix they produce to enable it to become mineralized. The final phase involves the invasion of the cartilage by blood vessels and the apoptotic death of these hypertrophic chondrocytes, leaving a space that becomes bone marrow. A group of cells that have surrounded the original cartilage differentiate into osteoblasts.

2.4.3.1.1 Osteoblasts[253]

Osteoblasts are cells that are located along the bone surface. They are derived from mesenchymal stem cells, the commitment of which towards the osteoprogenitor lineage requiring the expression of specific genes and timely programmed steps of the synthesis of bone morphogenetic proteins and members of the Wnt pathways. The expression of several Runt related transcription factors is necessary for osteoblast differentiation. In the proliferation phase of differentiation, osteoblast progenitors (pre-osteoblasts) show alkaline phosphatase activity, and their transition to mature osteoblasts is characterized by the secretion of bone matrix proteins such as osteocalcin , bone sialoprotein and collagen type I. The mature osteoblasts undergo morphological changes, becoming large and cuboidal in shape. These cells have morphological characteristics of protein synthesizing cells, such as abundant rough endoplasmic reticulum and prominent Golgi apparatus.

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[251] Ferretti E and Hadjantonakis A-K, Mesoderm specification and diversification: from single cells to emergent tissues, *Current Opinion n Cell Biology,* 2019;61:110-6. doi:10.1016/j.ceb.2019.07.012.

[252] Tani S, Chung U-I, Ohba S, et al, Understanding paraxial mesoderm development and sclerotome specification for skeletal repair, *Experimental and Molecular Medicine,* 2020;52:1166-77. doi:10.1038/s12276-020-0482-1.

[253] Florencio-Silva R, da Silva Sasso GR, Sasso-Cerri E, *et al,* Biology of bone tissue: Structure, function, and factors that influence bone cells, *BioMed Research International*, 2015, 421746. doi:10.1155/2015/421746.

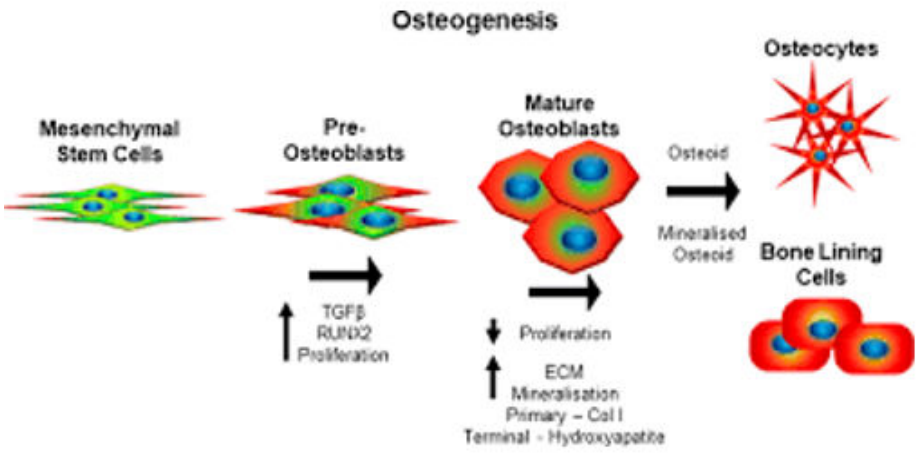

Figure 2.68. Sequence in osteogenesis and formation of osteoblasts.

2.4.3.1.2 Osteocytes

Osteocytes are derived from MSCs lineage through osteoblast differentiation. At the end of a bone formation cycle, a subpopulation of osteoblasts becomes osteocytes incorporated into the bone matrix. This process is accompanied by conspicuous morphological and ultrastructural changes, including the reduction of the round osteoblast size. The number of organelles such as rough endoplasmic reticulum and Golgi apparatus decreases, and the nucleus-to-cytoplasm ratio increases, which correspond to a decrease in the protein synthesis and secretion. Osteocytes comprise 90–95% of the total bone cells and are the most long-lived cells, with a lifespan of up to 25 years in humans. Cell-cell communication is particularly important in osteocytes and is achieved by interstitial fluid that flows between the osteocytes processes and canaliculi. Osteocytes act as mechanosensors; the interconnected network can detect mechanical forces, allowing the adaptation of bone to daily activity.

2.4.3.1.3 Osteoclasts

Osteoclasts are terminally differentiated multinucleated cells. They originate from mononuclear cells of the hematopoietic stem cell lineage, under the influence the macrophage colony-stimulating factor, and the RANK ligand, secreted by osteoblasts, osteocytes, and stromal cells. The RANKL/RANK/OPG system is a key mediator of osteoclastogenesis.

During bone remodeling osteoclasts polarize, and four types of osteoclast membrane domains can be seen: the sealing zone and ruffled border that are in contact with the bone matrix, and the basolateral and functional secretory domains, which are not in contact with the bone matrix. This polarization involves rearrangement of the actin cytoskeleton, The maintenance of the ruffled border is essential for osteoclast activity. An abnormal increase in osteoclast activity leads to bone diseases such as osteoporosis, where resorption exceeds formation causing decreased bone density and increased bone fractures. In some conditions including bone metastases and inflammatory arthritis, abnormal osteoclast activation can result in periarticular erosions and painful osteolytic lesions.

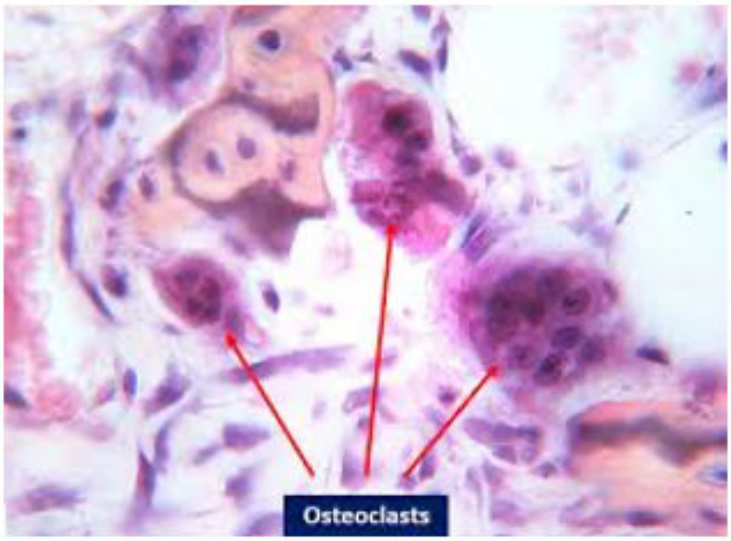

Figure 2.69. Osteoclasts.

2.4.3.1.4 Chondrocytes

The primary focus here is on chondrocytes in articular cartilage, the smooth viscoelastic tissue designed to distribute loads across the diarthrodial joints. This cartilage has an organized layered structure with superficial, middle, deep zone and calcified cartilage (Figure 2.70).

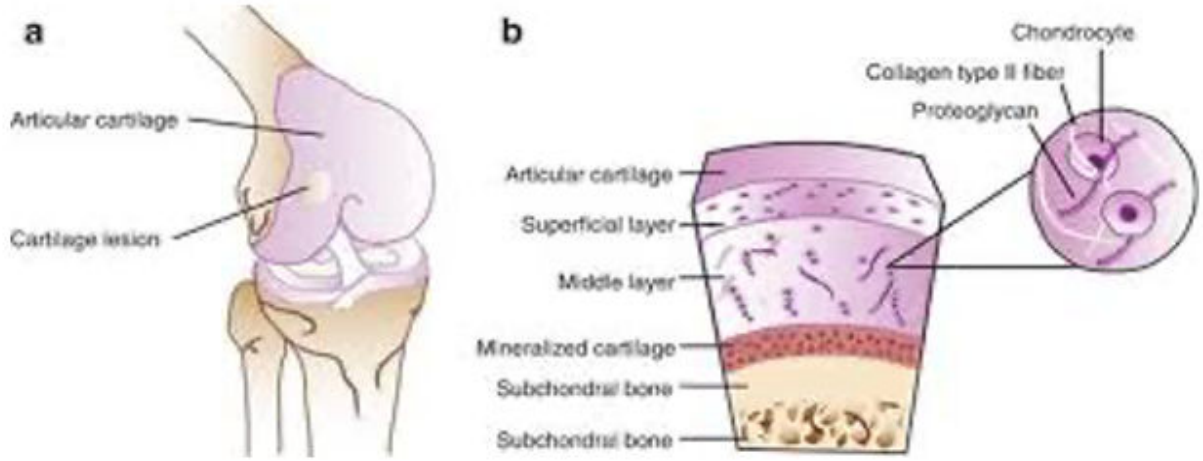

Figure 2.70. Articular cartilage and chondrocytes.

2.4.3.2 Fibroblasts[254] and Myofibroblasts[255]

A family of cells generally referred to as fibroblasts, is a heterogenous group that has the responsibility of synthesizing ad organizing ECM proteins, including collagens, elastin, laminins, fibronectin and some glycoproteins. As the mesoderm gives rise to the mesenchyme, mature fibroblasts develop and form the various types of connective tissue. A number of other cells, including endothelial cells, adipocytes and pericytes share the same mesodermal origin, and several transitions between cell types, for example epithelial to mesenchymal and endothelial to mesenchymal transitions, take place, accounting for the heterogeneity and plasticity of the fibroblast population. Primary fibroblasts arise from epithelial to mesenchymal transdifferentiation and give rise to all tissue resident fibroblasts. Resident quiescent fibroblasts are responsible for ECM homeostasis. Mesenchymal stromal cells are found in niches such as the bone marrow and peripheral blood and have a role in physiological organ regeneration. Some fibroblast sub-populations have role in wound healing and fibrogenesis.

Fibroblasts have an elongated spindle or stellate shape with many cytoplasmic projections. There is an abundance of rough endoplasmic reticulum and a large Golgi apparatus. The ECM is in constant communication with fibroblasts, which respond to both autocrine and paracrine signals. Matrix reorganization involves degradation and crosslinking enzymes, produced by fibroblasts, that are activated and regulated by pro-inflammatory cytokines and growth factors such as platelet-derived growth factor, granulocyte-macrophage colony-stimulating factor, epidermal growth factor, and tumor necrosis factor.

One important fibroblast transformation is that of fibroblast into the myofibroblast. Myofibroblasts are present in both healthy and pathologic tissues and contain features of fibroblasts and smooth muscle cells with additional distinguished features of nuclear membrane folds and long collections of microfilaments connecting to surrounding myofibroblasts and the extracellular matrix. These cells work in conjunction with vascular endothelial cells to form granulation tissue during times of wound healing.

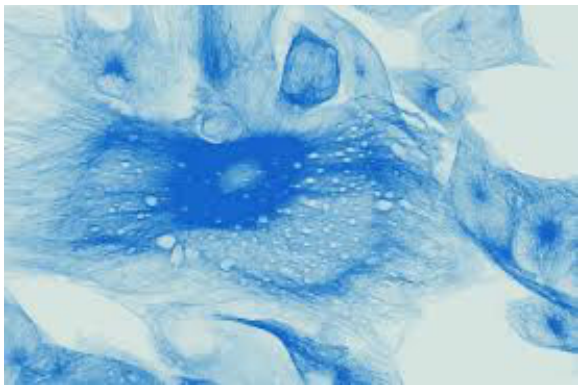

Figure 2.71. Representation of fibroblasts, (see Fibrobiologics.com).

Fibroblasts and myofibroblasts have potentially important roles in tissue formation in response to the implantation of medical devices and in the regeneration of tissues in tissue engineering. However, while the fibrous tissue that is normally generated (often referred to as scar tissue) provides for tissue continuity, it does not necessarily have the required properties for functional tissues. Fibroblasts are involved in all stages of wound repair, especially the later stages of fibrosis and remodeling. Fibrogenesis

[254] LeBleu VS and Neilson EG, Origin and functional heterogeneity of fibroblasts, *The FASEB Journal,* 2020;34:3519-36. doi:10.1096/fj.201903188R.
[255] Tomasek JJ, Gabbiani G, Hinz B, *et al,* Myofibroblasts and mechano-regulation of connective tissue remodeling, *Nature Reviews Molecular Cell Biology,* 2002;3:349-63. doi:10.1038/nrm809.

continues until fibroblasts and myofibroblasts are removed from the site through apoptosis. In normal processes, the wound healing cycle ends with the renewal of normal tissue structure and function. When fibroblast apoptosis occurs, prematurely non-healing or delayed healing can result. The prolonged inflammation delays apoptosis and continues ECM formation, with. high numbers of myofibroblasts in healed scar tissue.

The persistence of myofibroblasts within scar tissue contributes to hypertrophic scars, keloids, and fibromatoses. Hypertrophic scars are elevated and can slowly regress over time. Keloid scars have continued growth past wound edges and expand over time. Fibroblasts within keloids are an atypical subtype that is increasingly proliferative and activates surrounding fibroblasts. Dupuytren contracture is a hand deformity that slowly forces fingers into a bent position. The proliferation of fibroblasts arranged in cords, with nodules of myofibroblasts, and disordered collagen deposition causes disfiguration of the hand. Fibroblasts propagate this condition generating profibrotic cytokines in a prolonged manner subsequently leading to myofibroblast activation and continuous cytokine production.

2.4.3.3 Contractile Cells

Muscles operate through the performance of contractile cells. The contraction of muscles occurs by the activation of tension-generating sites within these cells, the precise characteristics of which depend on their location and the required function. Two variables are involved, the length and tension. The contraction may be isometric if the tension changes, but the length remains the same; it is isotonic if the tension remains the same as the muscle length changes. Many factors influence the relationship between these variables, including the length of time of the contraction.

The cytoplasm in a muscle cell is termed the sarcoplasm; the smooth endoplasmic reticulum is the sarcoplasmic reticulum; and the cell membrane is the sarcolemma. The embryonic precursors to muscle cells are the myoblasts. There are three types of muscle cell in humans. The myoblasts can fuse together to form multinucleated skeletal muscle cells in the process of myogenesis. These are long, threadlike and striated and are referred to as muscle fibers. Cardiac muscle cells, or cardiomyocytes, form the walls of the chambers of the heart and have a single central nucleus; like the skeletal muscle cells, these contain myofibrils and sarcomeres. Smooth muscle cells also have a single nucleus but have no myofibrils or sarcomeres and are not striated.

2.4.3.3.1 Skeletal muscle cells[256]

The primary functions of the skeletal muscle take place *via* its intrinsic excitation-contraction coupling process. As the muscle is attached to the bone tendons, the contraction of the muscle leads to movement of that bone that allows for the performance of specific movements. The skeletal muscle also provides structural support and helps in maintaining the posture of the body. Each muscle comprises multiple tissues, including blood vessels and lymphatics; thousands of contractile fibers are wrapped together within connective tissue sheaths. Each fiber is comprised of a number of myofibrils containing multiple myofilaments, the myofibrils being arranged in a striated pattern forming sarcomeres, the fundamental contractile unit of the muscle. Actin and myosin filaments are arranged to form distinctive bands on the skeletal muscle. The stem cells which differentiate into mature muscle fibers are known as satellite cells. Abnormalities in the skeletal muscles can give rise to several diseases include myopathy, paralysis, myasthenia gravis and urinary and/or bowel incontinence, Skeletal muscle and / or tendon ruptures can occur acutely in high-level athletes. Muscle cramps result in continuous, involuntary, painful, and localized contraction of an entire muscle group, individual single muscle, or select muscle fibers.

[256] Frontera WR and Ochala J, Skeletal muscle: a brief review of structure and function, *Calcified Tissue International,* 2015;96(3):183-95. Doi:10.1007/s00223-014-9915-y.

Generally, the cramp can last from minutes to a few seconds for idiopathic or known causes in healthy subjects or the presence of diseases.

2.4.3.3.2 Cardiomyocytes

Cardiomyocytes are tubular structures composed of chains of myofibrils, which have repeating sections of sarcomeres, Sarcomeres are composed of long proteins that organize into thick and thin myofilaments. As noted elsewhere in this book, myofilaments contain actin and myosin and slide past each other as the muscle contracts and relaxes, activating from the release of calcium from the sarcoplasmic reticulum when delivering an action potential to the muscle, in 'excitation-contraction coupling'. Cardiomyocytes contain many mitochondria to produce large amounts of adenosine triphosphate and myoglobin to store oxygen to meet the demands of muscle contraction. Unlike smooth and skeletal muscle, both of which require neural input for contraction, cardiac fibers have their own pacemaker cells, such as the sinoatrial node, that spontaneously depolarize at a consistent pace. Any insult or injury to cardiac muscle can have major consequences as cardiac muscle cells have minimal regeneration capabilities, a point discussed in several later sections.

2.4.3.3.3 Smooth muscle cells

Smooth muscle cells have several important functions in many parts of the body; the most important distinction between smooth and skeletal muscle is that it has the ability to be controlled involuntarily. The nervous system uses hormones, neurotransmitters, and other receptors to control smooth muscle spontaneously so that there is control over critical parameters such a urinary system function and the regulation of blood pressure and tissue oxygenation. Thus, smooth muscle can be found in the gastrointestinal tract, blood and lymphatic vessels, the urinary bladder, the male and female reproductive tracts, the respiratory tract and ciliary muscles and the iris of the eye. There are, in fact, two types of smooth muscle; single-unit smooth muscle consists of multiple cells connected through connexins that can be stimulated in a synchronous pattern from only one synaptic input. Connexins allow for inter-cellular communication, so that ions and molecules can diffuse between cells giving rise to calcium waves, permitting synchronous contraction. In multi-unit smooth muscle, each cell receives its synaptic input, giving much finer control. Multi-unit smooth muscle is found in the airways of the lungs and large arteries.

2.4.3.4 Circulatory System Cells[257]

Within the mesoderm, the hemangioblast is the common precursor to both endothelial cells and hematopoietic cells[258]. Upon activation by a variety of growth factors, the hemangioblasts swell to form blood island clusters, which can undergo one of two transitions, either flattening to become endothelium or differentiating to form hematopoietic cells.

2.4.3.4.1 Endothelial cells

Once endothelial cells have formed from the blood island clusters, they require further differentiation to direct them to either arterial or venous lineages, with exposure to mechanical / environmental cues and signaling pathways being involved. The vascular endothelium is a single layer of cells that line the luminal surface of blood vessels; they regulate the transport of many molecules between the blood and the interstitium of those vessels. During development and remodeling, arterial and venous vessels take on different characteristics. Arterial vessels have fewer branches and, because of the higher shear stresses imposed on them by the flowing blood, require significant external support. This is largely provided by

[257] Dyer LA and Patterson C, Development of the endothelium: An emphasis on heterogeneity, *Seminars in Thrombosis and Hemostasis*, 2010;36(3):227-35. doi:10.1055/s-0030-1253446.

[258] Xiong J-W, Molecular and developmental biology of the hemangioblast, *Developmental Dynamics,* 2008;237(5):1218-31. doi:10.1002/dvdy.21542.

vascular smooth muscle cells, which are separated from the endothelial cells by extracellular matrix proteins. Venous endothelial cells are supported by less smooth muscle cells and the walls are generally thinner. Not surprisingly, smaller blood vessels have some different structural features, as their functions are more closely related to molecular transport and metabolism. Endothelial cells do not only line the major blood vessels. They also line the interior of the heart, the endocardium, and valve leaflets. Many organs that have important functions of water, small molecule or hormone transport have a fenestrated endothelium, in which the endothelial cells have circular fenestrations, or windows that facilitate this transport.

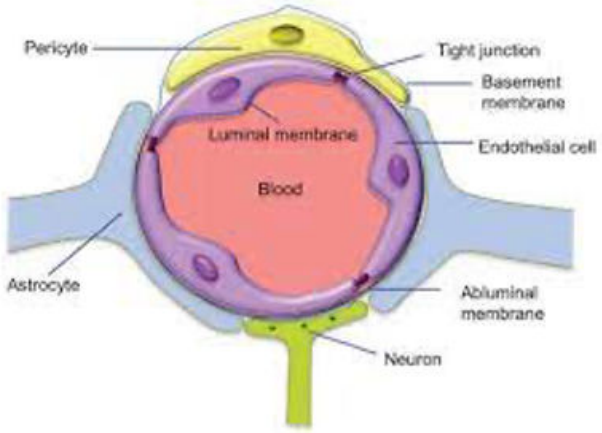

Figure 2.72. Endothelial cell and the blood brain barrier.

The blood-brain barrier also involves endothelial cells[259]. This barrier refers to the microvasculature of the central nervous system, in which the endothelial cells are very thin and are organized in such a way that they tightly regulate the movement of ions, molecules and cells between the blood and the brain. There are distinct luminal and abluminal membrane compartments which control movement to astrocytes and neurons, while tight junctions hold the endothelial cells together and limit the paracellular flux of molecules. The lymphatic systems also involve endothelial cells, a single layer of these cells lining lymph vessels; these are more permeable than vascular endothelial cells, allowing the removal of plasma proteins that have found their way into tissue spaces. The lymphatic vessels have layers of smooth muscle cells that generate pulsatile forces necessary for the movement of lymph, while there are overlapping intercellular junctions that open and close in response to interstitial pressure.

The endothelium is a very important factor in the development of several diseases, or conditions, and therapies that are directed towards endothelial cell function are potentially relevant to reconstructive processes. Atrial dysfunction is obviously a prime target here, where any abnormality in the release of molecules that regulate coagulation, platelet activity and vascular tone, such as nitric oxide, endothelin and prostacyclin, can have significant consequences, especially in the context of coronary artery disease. Hypertension decreases gap junction expression, decreasing cell-cell communication.

[259] Daneman R and Prat A, The blood-brain barrier, *Cold Spring Harbor Perspectives in Biology*, 2015;7:a020412, doi:10.1101:cshperspect.a020412.

2.4.3.4.2 Hematopoietic system[260]

In this brief section, mention is made of one of the key cell types in both embryogenesis and the maintenance of many cells, and the tissues that depend on them, throughout life; this is the hematopoietic stem cells. This mention is only brief since aspects of stem cells, including the hematopoietic stem cells, are potentially so important in critical regeneration / reconstruction technologies, that they are discussed in later sections in detail, and there is no need to pre-empt those discussions here. However, it is necessary to place the origin and function of these cells in context within this section on cells of the human body.

Hematopoiesis is the formation of blood or of blood cells in the body The hematopoietic system forms in the incipient embryo and different cell types emerge from the discrete anatomical niches that are present, and are able to change, both temporally and spatially. The hematopoietic stem cell is a multipotent stem cell that resides in the bone marrow and has the ability to form all the cells of the blood and immune system. It can self-replicate and differentiate into progeny of multiple lineages, so that hematopoiesis essentially describes the process of differentiating from these stem cells into mature, functional cell types of the blood lineages. There are two waves of development. Primitive hematopoiesis generates transitory cell populations, while definitive hematopoiesis follows, with the development of multipotent hematopoietic stem cells. The hematopoietic lineage is divided into two main branches: the myeloid and lymphoid arms, within which progenitor cells, the myeloblast and lymphoblast respectively, are differentiated. Myeloblasts are formed in the bone marrow and mature into white blood cells, the granulocytes, which are the precursor cells of eosinophils, neutrophils, and basophils. They are quite large cells, with irregular or round nuclei and some cytoplasm. Lymphoblasts are immature cells that mature into either B or T lymphocytes.

Dysfunction of the hematopoietic system can occur from a variety of genetic and acquired causes[261]. At a basic level, the stem cells may fail to produce healthy quantities of all blood cell types; this is pancytopenia which, while itself being not a disease, can give rise to several pathological conditions. Primary bone marrow failure syndromes result from a significant impairment of the hematopoietic stem cell pool resulting in various forms of marrow aplasia. Inherited germline mutations caused by intrinsic defects in the stem cells can give rise to conditions in many organs. Acquired marrow failure syndromes are caused by extrinsic damage, such as toxicity arising from exposure to chemicals. Also, antigen-driven immune mechanisms may induce marrow failure associated with lymphoproliferative or autoimmune conditions.

2.4.3.5 Blood and Immune System Cells

The origin of blood cells was outlined in the previous section. Here some details of the most relevant of these cells are presented.

2.4.3.5.1 White blood cells

White blood cells, referred to as leukocytes, participate in both the innate and humoral immune responses. They are classified as either granulocytes or agranulocytes, microscopic granules being present in the cytoplasm of the former but not the latter. Neutrophils, basophils, and eosinophils are all granulocytes. Neutrophils are 12 to 15 μm in diameter and have multi-lobed nuclei, giving rise to their alternative name of polymorphonuclear neutrophils. Neutrophils contain specific granules in the cytoplasm giving it a pale

[260] Julien E, El Omar R and Tavian M, Origin of the hematopoietic system in the human embryo, *FEBS Letters,* 2016;590:3987-4001. doi:10.1002/1873-3468.12389.

[261] Risitano AM, Maciejewski JP, Selleri C, *et al*, Function and malfunction of hematopoietic stem cells in primary bone marrow failure syndromes, *Current Stem Cell Research and Therapy*, 2007;2(1):39-52. doi:10.2174/157488807779316982.

pink color. When activated, the neutrophils migrate into the tissues in the process of diapedesis. These cells usually have life spans of a few days, and within connective tissue, they rapidly undergo apoptosis and are removed by macrophages. Eosinophils have a bi-lobed nucleus with large cytoplasmic specific granules. Basophils are 12 to 15 μm in diameter, have bi-lobed or S-shaped nuclei, and contain cytoplasmic specific granules (0.5 μm in diameter) that stain blue to purple. Agranulocytes consist of lymphocytes and monocytes. Lymphocytes are of varying sizes and have spherical nuclei. Lymphocytes are also subdivided into several groups, the major groups being the B and T lymphocytes. Monocytes are precursor cells for the mononuclear phagocytic system; this includes macrophages, osteoclasts, and microglial cells in connective tissue. These cells are 12 to 15 μm in diameter and have large, usually eccentric, nuclei that are indented or C- shaped. Leukopenia is a condition where the leukocyte counts are low; this can occur with viral infections and autoimmune conditions. On the other hand, leukocytosis is condition where the leukocyte counts (primarily neutrophils) are higher than normal, usually a sign of inflammatory response such as infection, including parasitic infections, or cancers such as leukemia.

2.4.3.5.2 Red blood cells

Red blood cells, also known as erythrocytes, are anucleated biconcave discoid shaped cells that are essential for the delivery oxygen from the lungs to the peripheral tissues, which is necessary for metabolic processes such as ATP synthesis, and for the return of carbon dioxide from the periphery to the lungs for elimination. The life-span of an erythrocyte is about 120 days. The cells have a phospholipid bilayer membrane that is supported by a cytoskeleton composed of a network of proteins, including actin and spectrin. This combination of a malleable protein network with the biconcave membrane allows the cells to respond to their surroundings through elastic / viscoelastic shape changes. This is immensely important since the cells must navigate through the very small blood capillaries, where inelastic deformation would normally result in irreversible property changes in the membranes, and premature ageing. These shape changes also allow for increased surface area when it is necessary to support gas exchange under stressed conditions. These observations, and many others about red blood cells were made by Leewenhoek way back in the seventeenth century "*The sanguineous globuls in a healthy Body must be very flexible and pliant, if they shall pass through the very small veins (which as I have said, are in the films where the flesh is as it were inwoven, and through which the blood circulates, that is to say passes from the arteries into the veins); and that, in their passage, they change into an oval figure, reassuming their former globosity when they come into a larger room, in accordance with their size, this being owing to their strong and frequent movement*"[262].

Anemia is the condition where the blood has insufficient healthy red blood cells, leading to reduced oxygen flow to tissues and organs, with resulting fatigue, skin pallor, shortness of breath, lightheadedness, dizziness. There are several different causes of anemia. Pernicious anemia is an autoimmune condition that restricts the absorption of vitamin B12. Iron-deficiency anemia occurs when there is insufficient iron to make hemoglobin. Sickle cell anemia is an inherited condition in which the normally flexible erythrocytes are changed into stiff and sticky sickle-shaped cells that block blood flow. In hemolytic anemia, the erythrocytes break down or die faster than usual; this can be a serious issue with any medical devices that come into long-term contact with blood, with abnormal hemodynamic conditions, either in the body or with extracorporeal devices. Aplastic anemia arises when stem cells in bone marrow do not make enough red blood cells, while in autoimmune hemolytic anemia, red cells are attacked by the immune system.

[262] Davis IM, "Round, red globules floating in a crystalline fluid" – Antoni van Leeuwenhoek's observations of red blood cells and hemocytes, *Micron,* 2022;157:103249. doi:10.1016/j.micron.2022.103249.

Figure 2.73 Red blood cells.

2.4.3.5.3 Platelets

Blood platelets are the smallest cells of the blood, at about 2 to 4 μm in diameter, but are more numerous (400,000 per cubic millimeter) than white cells. They lack a nucleus and are therefore incapable of mitosis. They tend to extrude hairlike filaments from their membranes, facilitating adhesion to each other. The function of platelets is directly concerned with hemostasis, that is the control of bleeding. When the endothelial surface lining of a blood vessel is damaged and compromised, platelets immediately attach to that surface and to each other, forming a tenaciously adherent mass, the effect of which is, (usually), to stop the bleeding and to form the basis of a blood clot, known as a thrombus. The mechanical effect of the blood clot on bleeding is significantly enhanced biochemically by the release of agents from granules that are present in the platelets. Platelets contain at least three types of granules, the α-granules, dense granules, and lysosomes. The α-granules are the most abundant, each being 200-500 nm in diameter and occupying about 10% of the platelet volume. They contain many proteins, including membrane-associated receptors such as P-selectin and soluble proteins such as fibrinogen and platelet factor 4. These molecules play active roles in clotting cascades and other, related processes. Lysosomes are less numerous, but they release various lysosomal enzymes that have important degradative functions.

Platelets are formed in the bone marrow by segmentation megakaryocytes, which are the largest cells of the marrow. The granular cytoplasm of the megakaryocyte divides into many small segments that break off and are released as platelets into the circulating blood. However, after about 10 days platelets are removed from the circulation and destroyed by reticuloendothelial cells. The rate of platelet production is controlled by the effect of thrombopoietin on the number and growth of megakaryocytes.

Platelet dysfunction may be inherited or acquired. Von Willbrand disease is the most common inherited platelet-related disorder. Acquired platelet disorders are usually caused by certain drugs such as aspirin and some non-steroidal anti-inflammatory agents. Diseases that can affect platelet function include cirrhosis, multiple myeloma and systemic lupus erythematosus.

2.4.3.5.4 Innate immune system cells

Macrophages reside in almost all organs, including the liver, brain, bones, and lungs. They have various specific functions in these organs. In the respiratory tract, alveolar macrophages are necessary for processing surfactants, and those in the gastrointestinal tract play an important role in the maintenance of homeostasis. However, it is their role in orchestrating the inflammatory response to foreign materials that it so important in the technologies of reconstruction since such materials are utilized in many of these reconstructive strategies, from implantable devices to tissue engineering. They are the cells that dominate the performance of so many of the technologies discussed in this book.

Macrophages have a fundamental defensive function against pathogens and have a significant role in the homeostasis through the disposal of waste materials and tissue repair[263]. Macrophages are able to present microbial antigens to T cells and are, therefore, effectors for cell-mediated immunity, being involved in the development and management of infectious diseases, cancers, and chronic inflammatory diseases. Phagocytosis plays a critical role in these processes[264]; this was elucidated by Metchnikoff in the late nineteenth century[265]. Metchnikoff identified phagocytic cells as possessing a primitive pluripotential autonomy that conferred on them the ability to 'eat' and then 'feed' other cells by their dual capacity to ingest particulate nutrients and move throughout the organism. Phagocytes continued to 'eat', but with a regulative function of maintaining the integrity of the organism by protecting the animal from foreign invaders or clearing the body of unwanted cellular debris, becoming the brokers of pathological inflammation, by engulfing and killing bacteria, congregating around foreign bodies, and appearing at wounds. By the 1890s, his phagocytic theory dominated the debates between himself and those who advocated primacy of humoral immunity as the key to host defense.

Phagocytosis is the process of ingesting and eliminating particles that are larger than around 0.5µm in diameter. Although many cells have this capability, it is only a few, which are described as professional phagocytes, that can do this with high efficiency; macrophages are the pre-eminent cells of this group. A target particle, whether that be a microorganism, a dead cell or biomaterial-derived entity, is internalized within a distinctive organelle known as a phagosome. As the phagosome mature through structural changes to its own membrane, it fuses with lysosomes, becoming a phagolysosome; this new organelle is rich in enzymes that are capable to degrading biological material.

Macrophages are classified as being either M1 or M2 types[266] (although there are subsets of these which make the situation a little more complex). M1 activity inhibits cell proliferation and causes tissue damage while M2 activity promotes cell proliferation and tissue repair. The names M1 and M2 were chosen because M1 and M2 macrophages promote Th1 and Th2 responses, respectively. The significance is that T lymphocytes are a major source of cytokines. There are two subsets of T lymphocytes, distinguished by their cell surface molecules. One group of T lymphocytes are also known as helper T cells, these being the most prolific cytokine producers. This subset can be further subdivided into Th1 and Th2. The relevance of this so-called macrophage polarization is discussed later in the context of inflammatory responses and biocompatibility.

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[263] Hirayama D, Iida T and Nakase H, The phagocytic function of macrophage-enforcing innate immunity and tissue homeostasis, *International Journal of Molecular Sciences*, 2018;19:92. doi:10.3390/ijms19010092.

[264] Uribe-Queroi E and Rosales C, Phagocytosis: Our current understanding of a universal biological process. *Frontiers in Immunology*, 2020;1:1066. doi:10.3389/fimmu.2020.01066.

[265] Tauber, A. Metchnikoff and the phagocytosis theory. *Nature Reviews Molecular Cell Biology, 2003;* 4, 897–901, doi:10.1038/nrm1244.

[266] Mills CD, M1 and M2 macrophages: Oracles of health and disease, *Critical Reviews in Immunology*, 2012;32(6):463-88. doi:10.1615/critrevimmunol.v32.i6.10.

One significant attribute of macrophages is their ability to fuse with each other to form multinucleated giant cells. Based on their morphology and functional characteristics, there are in general three types of multinucleated giant cells, the osteoclasts, Langhans giant cells and foreign body giant cells*;* the latter appear only under pathological conditions and are found in immune reactions against foreign materials, including implants. They may reach 1 mm in diameter, having an irregular shape and containing hundreds of nuclei throughout the cytoplasm.

Natural killer (NK) cells constitute a separate lymphocyte lineage of the innate immune system that have both cytotoxic and cytokine-producing effector functions[267]. They are active in, and control responses to, several types of tumors and microbial infections. They have a regulatory function with respect to interactions with macrophages, dendritic cells, T cells and endothelial cells.

2.4.3.5.5 Cells of adaptive immunity[268]

The concept of adaptive immunity arose from the work of various European scientists on immunological memory, vaccinations and the entities that became known as antibodies. In 1890, von Behring and Kitasato published their discovery of tetanus and diphtheria antitoxins, where the administration of gradually increasing doses of the toxoids in animals stimulated the production of antitoxins, and that these compounds, produced in live animals, were able to immunize other animals. The work faltered on the difficult technology of quality control during the production of these sera. In 1891, Paul Ehrlich was able to develop effective immunization protocols, and proposed the use of horses for the commercial production of the serum. A few years later, in attempting to explain 'immunity', Ehrlich formulated his 'side-chain theory' [269], which postulated that cells present on their surface a set of side-chains which might have a molecular structure that allowed binding with a specific toxin corresponding to diphtheria, tetanus, or some other microorganism. This strictly specific binding between the toxin and the side-chain, would mean the cell lost its normal function, which in turn would trigger the production of additional side-chains, many of which would be released into the blood stream. These would then act as antitoxins upon binding to the toxin present in the blood, preventing the toxin from binding to other cells in the organism. In 1900, Ehrlich introduced the term 'receptor' as a substitute for the term 'receptive side-chain', and the antitoxin became known as an antibody.

As far as cells of adaptive immunity are concerned, lymphocytes have already been mentioned in the section on white blood cells; these are the main effectors of the adaptive immune response. There are two types, the T lymphocytes, which mature in the thymus and the B lymphocytes, which arise in the bone marrow. Both types traffic to secondary lymphoid organs, such as the lymph nodes and spleen, which is where they capture antigens from lymph and blood. The adaptive immune responses originate in these areas. In order for the adaptive immune response to work efficiently, antigenic molecules on microbes or other entities must be processed into a form that is recognizable by the immune cells, and this is achieved by so-called antigen-presenting cells[270]. Many cells can operate in this way, but only a few have this as a major function, which include B-lymphocytes and macrophages and, especially, dendritic cells; these are referred to as professional antigen presenting cells. Dendritic cells are the major antigen-presenting cells in humans[271]. They do not destroy pathogens directly but communicate the presence of pathogens to the

[267] Vivier E, Tomasello E, Baratin M, *et al,* Functions of natural killer cells, *Nature Immunology,* 2008;9(5):503-8. doi:10.1038/ni1582.

[268] Bonilla FA and Oettgen HC, Adaptive immunity, *Journal of Allergy and Clinical Immunology,* 2010;125:S33-40. doi:10.1016/j.jaci.2009.09.017

[269] Bosch F and Rosich L, The contributions of Paul Ehrlich to pharmacology: A tribute on the occasion of the centenary of his Nobel Prize, *Pharmacology*, 2008;82(3), 171-9, doi:10.1159/000149583.

[270] Eiz-Vesper B and Schmetzer H, Antigen-presenting cells: potential of proven and new players in immune therapies, *Transfusion Medicine and Hemotherap*y, 2020;47:429-31. doi:10.1159/000512729.

[271] Mellman I, Dendritic cells: master regulators of the immune response, *Cancer Immunology Research,* 2013;1(3):145-9. doi:10.1058/2326-6066.CIR-13-0102.

adaptive system in order to initiate a long-lasting antigen-specific response. They partially degrade pathogen-derived proteins to generate pathogen derived peptides, which form complexes with other molecules that are presented to the relevant T cells.

One further cell of relevance here is the mast cell[272]. These cells originate from the pluripotent progenitor cells of the bone marrow and they mature under the influence of signals provided by the environment of the tissues where they will reside. They are present throughout the body but do not normally circulate in blood. Their main mechanism of action involves IgE-mediated allergic reaction. When an antigen comes into contact with a mast cell, it activates the release of granules, which have different effects in different parts of the body, but these primarily involve stimulation of both innate and adaptive immune responses. The molecules released upon activation include histamine, heparin and many cytokines and chemokines. The most common sites of the body where mast cell responses are prominent are the mucosa of the respiratory tract, dealing with airborne allergens, and of the gastrointestinal tract, dealing with food born allergens.

2.4.3.6 Reproductive System Cells

In this section, the main functional cells of the reproductive system are introduced, covering the germ cells are the basis of all sexually reproducing organisms, and the cells of the supporting structures that assist in this function.

2.4.3.6.1 General characteristics of germ cells

Germ cells are set aside from all somatic cells of the embryo. They tend to form at the fringe of the embryo and migrate through the developing somatic tissues toward the emerging gonad, which is defined as that part of the reproductive system that produces and releases eggs and sperm. Once in the gonad, germ cells acquire sex-specific morphologies, with the ability to undergo meiosis to generate egg and sperm. The germline escapes the mortality that all somatic cells ultimately face, through a combination of transcriptional repression of somatic differentiation during embryogenesis, cell-to-cell signaling between the somatic and germ cells that governs germ cell proliferation, maintenance, and differentiation and conserved RNA regulatory networks within germ cells that prevent somatic transdifferentiation coordinate germline-specific processes.

2.4.3.6.2 Human eggs and oocytes

An egg can give rise to every cell type in the adult organism, although it is itself a highly specialized cell. The eggs of humans, and most animals, are giant single cells, containing all the materials needed for initial development of the embryo. The mammalian embryo can start to grow early by taking up nutrients from the mother via the placenta. Eggs are typically spherical or ovoid, with a diameter of about 0.1 mm in humans. A developing egg is called an oocyte, the development of which is referred to as oogenesis. Primordial germ cells migrate to the forming gonad to become oogonia, which proliferate by mitosis for a period before differentiating into primary oocytes. At this stage, the first meiotic division begins and the DNA replicates so that each chromosome consists of two sister chromatids. One of the final steps in oogenesis is maturation, defined as a re-entry into meiosis that occurs just prior to ovulation and subsequent fertilization. Oocytes within the ovary are arrested in prophase I of meiosis until the gonadotropins, follicle-stimulating hormone and luteinizing hormone stimulate follicular growth and development, which then triggers the resumption of meiosis up to metaphase II. Oocytes are then held in meiotic arrest until fertilization, when meiosis is completed.

[272] Krystel-Whittemore M, Dileepan KN and Wood JG, Mast cell: a multifunctional master cell, *Frontiers in Immunology,* 2016;6:620. doi:10.3389/fimmun.2015.00620.

In the context of regenerative technologies, it is noted that the cytoplasm of an egg can reprogram a somatic cell nucleus under some circumstances so that the nucleus can direct the development of a new individual. This is the basis of cloning, where the nucleus of an unfertilized egg may be deliberately destroyed and replaced with the nucleus of an adult somatic cell. This is briefly discussed elsewhere.

2.4.3.6.3 Human sperm

Spermatogenesis involves the origination and development of sperm cells within the male reproductive organs, the testes, which are composed of many tightly coiled thin seminiferous tubules. The immature sperm cells themselves are produced from stem cells, which are composed almost entirely of nuclear material, within the walls of the tubules. Sperm cells are continuously produced by the testes, but not all areas of the seminiferous tubules produce sperm cells at the same time. There are intermittent resting phases in the sperm cell growth process, and it may take many weeks to reach final maturation. The human sperm has a head and motile tail. In the head is a specialized secretory vesicle, the acrosomal vesicle, which contains hydrolytic enzymes that help the sperm to penetrate the outer coat of an egg. On contact with an egg, the contents of the vesicle are released by exocytosis in the acrosome reaction, which may also expose specific proteins that help bind the sperm tightly to the egg coat. The motile tail is a long flagellum, whose central core emanates from a basal body near the nucleus. Flagellar movement is driven by motor proteins, which use the energy of ATP hydrolysis, the ATP being generated by highly specialized mitochondria in the anterior part of the tail.

2.4.3.6.4 Sertoli cells

Sertoli cells are present in the seminiferous tubules of the testes. They help to facilitate spermatogenesis, and the production of viable sperm. Sertoli cells also secrete a range of vital molecules, include androgen binding protein, inhibin B, and activin, which support spermatogenesis directly or indirectly *via* a hormonal negative feedback system. The cells also respond to pituitary hormones such as follicle-stimulating hormone to begin the process of spermatogenesis, supplementing the adjacent spermatogonia. The Sertoli cell structure forms tight junctions and connective adhesion molecules with neighboring Sertoli cells to induce local sequestering of testosterone. This is the basis of the blood-testes-barrier, which provides many necessary conditions for appropriate spermatogenesis, including ion regulation, testosterone concentration, immune system evasion, and barrier protection. Sertoli cells are so important that their mere absence in testes can lead to infertility in adult males even though the production of sperm is normal. They also secrete Mullerian Inhibiting Factor, a compound that helps to prevent the development of female sex organs following the determining of the testes embryologically.

2.4.3.6.5 Leydig cells

Leydig cells, also known as testicular interstitial cells, are located between seminiferous tubules, which, as we have seen, contain Sertoli and germ cells; these three cell types maintain spermatogenesis, control hormonal regulation, and affect some sexual characteristics, being under hormonal regulation by the hypothalamic-pituitary axis. Leydig cells are the primary source of testosterone or androgens in males. With the development of male embryos, Leydig cells produce androgens, inducing Wolffian duct development into male urogenital structures. Infantile Leydig cells are replaced by adult Leydig cells, maintaining androgen through life.

2.4.3.6.6 Granulosa and Peg cells

Granulosa cells are critical to female reproduction. Under the control of pituitary glands, they produce hormones, including estrogen and progesterone, and support ovarian follicles. From the start of a menstrual cycle right through to ovulation, the released follicle-stimulating hormone directs the production of estrogen sex hormones. There are two types of granulosa cells: cumulus cells surround the oocyte and give it nutrients, staying with the egg as it passes through the fallopian tube, while mural granulosa cells line the walls of the follicles and surround the part of the follicle filled with fluid.

Peg cells are non-ciliated epithelial cells within the Fallopian tubes. They secrete molecules that facilitate passage of the egg and protect sperm.

2.4.4 Cells Derived From Ectoderm (Outer Layer of Embryo)

Cells derived from the ectoderm mainly reflect the structure and importance of the neural crest[273]. During embryo development, the neural tube is the precursor to the central nervous system. The initial neural groove deepens as the neural folds become elevated and coalesce in the middle line and convert the groove into the closed neural tube. The ectodermal wall of the tube is the rudimentary nervous system.

The neural tube then develops in two ways. In primary neurulation, the ectoderm divides into three cell types, the internally located neural tubes, the externally located epidermis and the neural crest cells, which develop between the neural tube and epidermis but then migrate to new locations. In secondary neurulation, the cells of the neural plate form a structure that migrates inside the embryo and hollows to form the tube.

The neural crest cells originate at the dorsal most region of the neural tube. Thus, both the prospective neural plate and the prospective epidermis contribute to the neural crest. The neural crest cells migrate extensively to generate many differentiated cell types, including the neurons and glial cells of the sensory, sympathetic, and parasympathetic nervous systems, the epinephrine-producing (medulla) cells of the adrenal gland, cells of the epidermis, and many of the skeletal and connective tissue components of the head.

Cells of the cranial (cephalic) neural crest migrate dorsolaterally to produce the craniofacial mesenchyme that differentiates into the cartilage, bone, cranial neurons, glia, and connective tissues of the face. These cells give rise to thymic cells, odontoblasts of the tooth primordia, and the bones of middle ear and jaw. Cells of the trunk neural crest follow one of two major pathways. Neural crest cells that become the pigment-synthesizing melanocytes migrate into the ectoderm and continue toward the ventral midline of the belly. The second pathway takes the cells ventrolaterally through the anterior blocks of mesodermal cells, that will differentiate into the vertebral cartilage of the spine. Some of the trunk neural crest cells remain and form the dorsal root ganglia containing the sensory neurons. Other cells that continue ventrally to form the sympathetic ganglia, the adrenal medulla, and the nerves that surround the aorta. Cells of the vagal and sacral neural crest generate the parasympathetic (enteric) ganglia of the gut. The cardiac neural crest is located between the cranial and trunk neural crests and can develop into melanocytes, neurons, cartilage, and connective tissue. This region also produces the entire muscular connective tissue wall of the large arteries as they arise from the heart, as well as some of the septa in the circulatory system.

2.4.4.1 Nervous System Cells

2.4.4.1.1 Central nervous system

A description of these cells has been included in earlier section on the brain and spinal cord and so their mention here will be brief. This system has two categories of cell, the neuron and the glial cells. There are several different types of glial cell.

[273] Theveneau E, The neural crest, *Development,* 2013;140(11):2247-51, doi:10.1242/dev.091751.

Astrocytes[274] are specialized glial cells; they are present in large numbers and contiguously cover the entire central nervous system, where they exert many essential complex functions, including primary roles in synaptic transmission and information processing by neural circuit functions. There are two main subtypes, protoplasmic or fibrous. The former are found throughout all gray matter, with several stem branches that give rise to finely branching processes in a uniform globoid distribution. Fibrous astrocytes, on the other hand, are found throughout all white matter and have many long fiber-like processes. Astrocytes processes enfold blood vessels within the brain, and ensheath single as well as groups of synapses, so that they are well-positioned to regulate extracellular concentrations of ions and neurotransmitters. When neurons fire action potentials, they release K^+ ions into the extracellular space. They take up K^+ at sites of neuronal activity, specifically synapses, and release it at distant contacts with blood vessels. K^+ channels belong to the largest super-family of ion channels, many of which are Ca^{2+}-activated. Astrocytic K^+ and Ca^{2+} are involved in many fundamental pathophysiological functions, including mitochondria biogenesis, cell survival, apoptosis, vascular tone, neurotransmitter release, and gene expression. After CNS injury, the accumulation of ions and water in astrocytes can lead to severe brain swelling. In addition to ion homeostasis, astrocytes play a critical role in synapse formation and elimination. Once formed, synapses continue to mature and are later involved in the elimination of a large proportion of synapses, a process that is accompanied by the growth and strengthening of surviving synapses. Astrocytes-derived molecules, such as thrombospondin and cholesterol, modulate synapse function. Astrocytes also have phagocytic functions, eliminating live synapses and clearing synaptic debris.

Oligodendrocytes have the principal function of producing myelin, the insulating sheath on the axons of nerve fibers. They have few cytoplasmic fibrils but a well-developed Golgi apparatus; they have a greater density of cytoplasm than astrocytes and a large number of microtubules in the processes. They are subdivided into interfascicular and perineural forms. The interfascicular oligodendrocytes are aligned in rows between the nerve fibers of the white matter. In gray matter, perineuronal oligodendrocytes are located in close proximity to the somata of neurons. The type of axon determines whether the myelination is loose or tight. In tight myelination, the oligodendrocyte wraps around a length of axon until the fiber is covered by several layers. Between segments of myelin wrapping are exposed sections called nodes of Ranvier, which are important in the transmission of nerve impulses.

Microglia cells[275] are the immune cells of the central nervous system. In a resting healthy brain, microglia are highly dynamic, actively surveying the brain parenchyma. They can rapidly respond to pathological insults, contributing to both pathogenesis and conferring neuronal protection. In addition, interactions between microglia and neurons shape neural circuit activity and they are involved in monitoring the integrity of synaptic function.

2.4.4.1.2 Peripheral nervous system

In addition to the neurons, the peripheral nervous system is comprised of four major types of glial cells. They are Schwann cells, satellite glial cells, enteric glial cells, and olfactory ensheathing cells.

Schwann cells myelinate peripheral nerves and serve as the primary glial cells of the peripheral nervous system, insulating and providing nutrients to axons. Myelination increases conduction velocity along the axon. Non-myelinating Schwann cells do not wrap axons, but provide trophic support and cushioning to the unmyelinated axons. Each Schwann cell makes up a single myelin sheath on a peripheral axon; numerous Schwann cells are needed to myelinate the length of an axon. Schwann cells are surrounded by

[274] Sofroniew MV and Vinters HV, Astrocytes: biology and pathology, *Acta Neuropathologica*, 2010;119(1):7-35, doi:10.1007/s00401-009-0619-8.

[275] Wake H, Moorhouse AJ and Nabekura J, Functions of microglia in the central nervous system--beyond the immune response, *Neuron Glial Biology*, 2011;7(1):47-53, doi:10.1017/S1740925X12000063.

a basal lamina and between adjacent myelin sheaths, there are gaps of approximately 1 micron. There is a concentration of voltage-gated sodium channels at the node.

Satellite glial cells[276] wrap around neuronal cell bodies, in most cases forming a complete envelope. They are found exclusively in peripheral ganglia, that is the sensory, parasympathetic and sympathetic ganglia, the latter two being part of the autonomic nervous system. The gap between these cells and the neuronal surface is about 20 nm, similar to that of the synaptic cleft, which allows for close mutual neuron–SGC interaction. These cells in sensory ganglia are activated during nerve injury and inflammation. The activation includes upregulation of glial fibrillary acidic protein, stronger gap junction-mediated coupling, increased sensitivity to ATP and increased cytokine synthesis and release. These changes appear to contribute to chronic pain by augmenting neuronal activity.

Enteric glia[277] are peripheral neuroglia that are associated with the cell bodies and processes of enteric neurons within the digestive tract, fulfilling homeostatic functions within this system. Enteric glia are one of the most dynamic signaling components of the ENS and fast, bidirectional communication between enteric glia and neurons regulates enteric reflexes and the communication between extrinsic and intrinsic neurons that innervate the digestive tract. They contribute to diverse disease processes, including neuroinflammation, cancer and infection. Both losses and gains of glial functions affect gastrointestinal pathophysiology.

2.4.4.2 Skin Cells

The skin is made up of three layers, the epidermis, dermis, and the hypodermis, which together made up of an intricate network that constitutes the initial barrier against injury, pathogens, UV light, and chemicals. The epidermis is the most cellular. Being comprised of different layers, the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. the latter being the most superficial part. There are four prominent cells in these layers.

2.4.4.2.1 Keratinocytes

Keratinocytes are the predominant cells in the deepest basal layer of the epidermis, constituting about 95% of the epidermal cells. Squamous keratinocytes are also found in the mucosa of the mouth and esophagus, and in the corneal, conjunctival and genital epithelia. Within that basal layer, the cells divide, giving rise to transient amplifying cells, which divide further as they migrate towards the surface. The differentiating cells produce proteins, especially keratins, that provide the integrity of the outermost layer, the stratum corneum. The keratinocytes in the stratum corneum are dead squamous cells that no longer multiply, creating the tough outer layer of skin. The keratinocytes are maintained at different stages of differentiation throughout the epidermis, being responsible for forming tight junctions with the nerves and keeping Langerhans cells of the epidermis and lymphocytes of the dermis in place. In addition to their physical role, keratinocytes are immunomodulators, stimulating inflammation and activating Langerhans cells in response to injury. Langerhans cells serve as antigen-presenting cells when there is a skin infection and are the first cells to process microbial antigens entering the body from a breach of the skin.

2.4.4.2.2 Melanocytes

Melanocytes comprise a heterogeneous group of cells, which are present in the skin but also elsewhere. Not only are they in the epidermis and iris, but also in the inner ear, the nervous system and the heart. In the skin they are found between cells of the stratum basale. Their major function is to produce melanin,

[276] Hanani M and Spray DC. Emerging importance of satellite glia in nervous system function and dysfunction. *Nature Reviews Neurosciences, 2020;* **21**: 485–98). doi:10.1038/s41583-020-0333-z.

[277] Seguella L and Gulbransen BD, Enteric glial biology, intercellular signaling and roles in gastrointestinal disease, *Nature Reviews Gastroenterology Hepatology*, 2021;18(8):571-87, doi:10.1038/s41575-021-00423-7.

which is protective against UV radiation. Melanin is produced during the conversion of tyrosine to DOPA by the enzyme tyrosinase and travels from cell to cell by mechanisms that involve the long processes which extend to the neighboring epidermal cells. Melanin granules from melanocytes are transferred *via* the long processes to the cytoplasm of basal keratinocyte. The life cycle of melanocytes consists of several steps including lineage specification from melanoblasts, in the embryonic neural crest, their migration, proliferation and differentiation into melanocytes, maturation of melanocytes and transport to keratinocytes and eventual cell death. Several populations of neural crest cells (cranial, dorsal trunk, ventral trunk) give rise to the melanocytes of the skin.

2.4.4.2.3 Langerhans cells

Langerhans cells are dendritically shaped cells, which are located in the suprabasal layer of the squamous epithelia of epidermis. They constitute only 3% of the cell population in epidermis and act as antigen presenting cells during initiation of immune responses. They are part of the mononuclear phagocytic system; they contain Birbeck granules as cytoplasmic organelles. These cells take up antigens in the skin and transport them to the lymph nodes.

2.4.4.2.4 Merkel cells

Merkel cells are oval-shaped epidermal cell in stratum basale, directly above the basement membrane, which serve a sensory function as mechanoreceptors. They are most populous in fingertips, though also found in the palms, soles, oral, and genital mucosa. They are bound to keratinocytes by desmosomes and contain keratin filaments; their membranes interact with nerve endings in the skin.

2.4.4.2.5 Dermis and Hypodermis

Fibroblasts are the primary cells within the dermis and the hypodermis, but macrophages, mast cells, and adipocytes are also present.

2.4.4.3 Oral cells

2.4.4.3.1 Odontoblasts[278]

Odontoblasts are columnar cells at the periphery of the dental pulp. They derive from ectomesenchymal cells originated by migration of neural crest cells during the early craniofacial development. A conspicuous cell process arises from the cell body and penetrates into the mineralized dentine. Odontoblasts deposit new layers of dentine throughout life and can form a reparative (or sclerotic) dentine in response to dental caries and other external factors. They are responsible for the maintenance of dentinal tubules and dentinal fluid and also act as cellular component of the dental temperature sensing system either by sensing temperature changes directly or by detecting hydrokinetic forces of fluid movement in the tubules.

2.4.4.3.2 Cementoblasts

Cementoblasts form from the follicular cells around the roots of teeth, with form and function somewhat similar to osteoblasts. Cementoblasts lie within lacunae, which have canaliculi that are oriented towards the periodontal ligament. They contain processes that allow diffusion of nutrients from the vascularized ligaments, but have no nerves. The cementoblasts are essentially entrapped in the cementum and once entrapped are no longer able to secrete cementum. In healthy teeth and periodontal tissues, however there are always cementoblasts along the outer covering of the ligament.

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[278] Arana-Chavez VE and Massa LF, Odontoblasts: the cells forming and maintaining dentine, *International Journal of Biochemistry and Cell Biology*, 2004;36(8(:367-74, doi:10.1016/j.biocel.2004.01.006.

2.4.4.3.3 Ameloblasts

Enamel is formed by ameloblasts, which are differentiated dental epithelial cells. Proliferating dental epithelial cells first differentiate into inner enamel epithelium, then into pre-ameloblasts, presecretory ameloblasts then into the secretory ameloblasts which synthesize and secrete enamel matrix proteins. These proteins self-assemble to form a matrix, which mineralizes as the ameloblasts continue to differentiate. Ameloblasts commit to apoptosis before a tooth erupts so that functional enamel in human teeth is acellular.

2.4.4.3.4 Other oral cells

There are other cells within dental and parodontal tissues but these are generally not specific to these tissues, and are covered elsewhere in this text. For example, the vast majority of cells in the periodontal ligament are fibroblasts. Dental pulp is a highly vascularized and innervated mass of connective tissue that resides within the pulp chamber and contains fibroblasts, odontoblasts, macrophages, mast cells, and plasma cells.

2.4.4.4 Other Cells

The descriptions of cells in this section have largely concentrated on specialized cells whose functions control the performance of many types of tissue. There are several other immensely important cell which are not included here because they constitute the functional parts of major organs, such as the liver and kidneys, have been described in detail within those sections that cover these organs. Other cells that have both generic and specific characteristics but not linked to any particular tissue, including stem cells and cancer cells, are also discussed in more relevant places.

2.5 REVIEW OF OPPORTUNITIES FOR RECONSTRUCTION

This section is intended to place in perspective the current status (around 2024) of reconstructive technologies, and opportunities for the future, largely based on the discussions of the present chapter. It was tempting to summarize the components of the body, from the major organs down to small molecules, and identify the relevant technologies that exist. That approach, however, would not allow an overarching picture to emerge. I decided that the better approach would be to identify, generically, the causes, or origins, of the underlying conditions that motivate reconstruction, and examine the pattern that develops. I have identified the relevant technologies, and their abbreviations as follows:

- Transplantation Tx
- Tissue graft CTG
- Implantable device MedDev
- Artificial organ / assist AO
- Tissue engineering Regen
- Cell therapy CT
- Gene therapy GT
- Gene editing GE
- Pharmaceutical Rx

I then identify the generic causes of the conditions, as follows;

- Hereditary / genetic conditions
- Biophysical disruption
- Biochemical abnormalities
- Biomechanical disruption

- Immune system dysfunction
- Metabolic conditions
- Trauma
- Infectivity
- Mutagenicity / carcinogenicity
- Psychological conditions
- Non-specific old age

Naturally many of the conditions have more than one cause. It will be noted that I do not include socio-economic factors as causes, such as poverty, malnutrition and obesity since it is more relevant in the present context to consider the physiological aspects as the drivers of reconstruction needs.

The following figure shows my interpretation of the status of our reconstructive technologies with respect to these underlying causes. I have tried to indicate where these individual technologies are in terms of maturity.

(To be completed, Q1, 2024)