INTRODUCTION

> "The 'Red Death' had long devastated the country. No pestilence had ever been so fatal, or so hideous. Blood was its Avatar and its seal — the redness and the horror of blood. There were sharp pains, and sudden dizziness, and then profuse bleeding at the pores, with dissolution. The scarlet stains upon the body and especially upon the face of the victim, were the pest ban which shut him out from the aid and from the sympathy of his fellow-men. And the whole seizure, progress, and termination of the disease, were the incidents of half-an-hour."
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> *The Masque of the Red Death*
> *Edgar Allan Poe 1809-1849*

Simply a terrifying Gothic tale conjured from the deep recesses of a tormented mind? No, not necessarily. Such diseases exist, albeit thankfully rare, within the group of haemorrhagic fevers caused by viral infection. In the more subdued language of modern science, "they are characterised by an insidious onset of influenza-like symptoms followed, in severe cases, by a generalised bleeding diathesis (spontaneous bleeding), encephalopathy and death" (D Cummins, 1991, *Blood Rev.*, **5**, 129–137). The time-scale may be several days not half-an-hour but, with poetic licence, the visible oozing of blood from mucous membranes, the feverish state of mind and the general disintegration of the body induced by massive internal haemorrhage and its sequelae are correct. The latter aspect of bodily destruction can be exemplified by another disease, necrotizing fasciolitis, an as yet uncommon soft-tissue infection usually caused by toxin-producing virulent bacteria such as ß-haemolytic Streptococci, and which is characterised by widespread cellular necrosis. "It starts typically with a purple lesion of the skin, followed by necrotizing fascial infection with secondary necrosis of the overlying skin (and underlying muscle) and rapid progression to septic shock and multiorgan failure." (T Eugester *et al*., 1997, *Swiss Surg*., **3**, 117–120). "... microorganisms rapidly spread along the fascial plane, causing necrosis of the fascia, overlying skin, and vasculature. Septicemia and systemic toxic effects may lead to death within as short a time as 24 to 96 hours." (PB Gillen, 1995, *J Wound Ostomy Continence Nurs*., **22**, 219–222). The microbes produce hyaluronidase, an enzyme which digests hyaluronic acid and thus liquifies the ground substance of the connective tissue, making it easier to advance and clearing the way for the invasion, spreading out in waves from the point of infection.

Living Systems

In the primordial soup, energy from solar radiation and electromagnetic discharge was trapped by small molecules, exciting them and giving them sufficient energy to interact and eventually create larger and more complex organic molecules. As this process persisted, aggregation and coalescence of large molecules into ordered functional systems facilitated the continuum from chemical to biological evolution.

With increase in molecular complexity came the ability to self-assist chemical reactions. Pockets enfolded within large molecules could impose advantageous electronic surroundings and help guide reactive entities together, acting as catalysts to ensure chemical reactions occurred against unfavourable odds. As these enzymes became more efficient and reliable, self-replicating macromolecules evolved. These were probably only poor copies at first, but as the enzymes refined, more exact replicates would result. However, without a barrier to protect these groups of interactive macromolecules from continual chemical assault, little overall progress could be made. It was necessary to maintain constant and favourable conditions, to isolate a small section of the territory.

The development of molecules possessing areas which both attracted and repelled the ionic aqueous milieu permitted their alignment and integration, so that their hydrophilic areas were always presented to the ubiquitous watery habitat whereas their hydrophobic ends turned inwards towards themselves, repelled from the water, forming a sphere. Like primitive fat droplets, the inside was protected from changes occurring on the outside; primordial membranes emerged. Characteristics of these primitive membranes allowed the selective flow of molecules, enabling certain components to be concentrated inside and other components to be actively excluded. Thus, for the first time the medium in which chemical reactions could take place could be controlled and optimised. If the interactive macromolecules could become trapped within a primitive membrane, a progenitor cell would be formed.

The Cellular Machine

All living entities are comprised of cells. The cell is the basic living unit of all organisms. Smaller systems exist, for example viruses, but these can only become alive when they enter and usurp the cell's normal function.

Most cells possess a nucleus which is bound within a membrane and contains a complex macromolecule, DNA, which encodes instructions for the construction of the complete cell. Mutations in the DNA, which may arise for many reasons, can lead to disruption of cellular function and the production of abnormal and malfunctioning components. Within the cytoplasm, outside the nucleus but bound by the cell membrane, lie many oraganelles. The endoplasmic reticulum is a series of phospholipid tubes or cavities which traverse the cytosol assisting in intracellular transport and containing enzymes which synthesize proteins. The Golgi apparatus, a specialised region of the endoplasmic reticulum, is responsible for the collection, modification and export of metabolic products from the cell, usually packaged into membraneous vesicles. Mitochondria are elongated peanut-shaped inclusions which have a double membrane. Whilst the outer membrane is smooth, the inner membrane is folded into numerous elongated projections housing a series of enzymes and linked protein complexes which transport electrons. This is the powerhouse of the cell and is concerned with the major catabolic processes of cellular respiration, whereby energy stored in metabolic fuels is made available. In addition, many cells, depending upon their particular specialist function, contain other inclusions such as lysosomes, centrioles and microtubular networks as well as droplets of fat, protein granules, and various crystalline substances.

Both synthesis of new cells and the maintenance of existing cellular function requires the expenditure of energy. It is a constant battle between cellular degradation, sometimes accelerated by the ingestion of toxins, and necessary repair. This energy comes from foods. In effect, the carbon atoms contained within the proteins, carbohydrates and fats eaten in our everyday diet are converted, in a controlled step-wise degradation, into carbon dioxide which is exhaled. Hydrogen atoms are converted to water and nitrogen is lost from the body as urea. The increments of energy released from these complex molecules as they are systematically disassembled is, via the assistance of the mitochondria, stored in high energy molecules called adenosine triphosphate (ATP). This ATP acts like a rechargeable battery, enabling the energy released by all these catabolic pathways to be stored and moved around the body to where it is required. Once the ATP has discharged its energy, it flows back to the mitochondria for recharging.

Routes of Entry

Toxins, or poisons, can enter the body by a number of routes. The more usual, and perhaps accidental, routes are called the "portals of entry". These comprise those areas of the body that are in constant contact with the external environment and over which we have little obvious control, unless special precautions are taken.

The skin is the largest organ of the body, composed of approximately 20 square feet of tough resilient tissue it forms the interface between ourselves and the outside world. Usually, although not always, the majority of our skin is covered with clothing but the face and hands, perhaps the arms and lower legs, may normally be exposed. The skin is waterproof and forms an effective barrier to many potentially noxious chemicals. However, certain lipid-soluble compounds may penetrate the skin readily, crossing the outer epidermis and entering the lower dermis with its direct access to the circulatory system. Entry may also be gained through abrasions in the skin surface or via hair follicles, nail beds and sweat glands which traverse the epidermis.

Many substances enter the body via the mouth, taken in deliberately or accidentally with food and drink. A small number of compounds, such as nicotine, may be absorbed directly across the thin membranes lining the nose and mouth and rapidly enter the circulatory system. Others have to travel down into the stomach and small intestine where, dependent upon their physicochemical properties, they are absorbed across membranes and enter blood or lymphatic vessels. The blood draining from the stomach to the top of the rectum enters a special series of blood vessels called the hepatic portal system which delivers the blood directly to the liver. The liver is initially important in protection against toxicity and, unlike other tissues and organs in the body, can regenerate after damage or surgical resection. The liver contains a range of enzymes which are able to metabolise chemicals usually, but not always, decreasing their potential activity thereby limiting their ability to interfere with cellular function, and also increasing their water solubility making it easier to actively remove them from the body via the kidneys into the urine. Thus, because of its unique anatomical position between the incoming blood from the gastroinestinal tract and the outgoing blood into the body's systemic circulation, the liver is able to act as a filter, removing or deactivating potentially toxic compounds. Fat soluble compounds may enter the lymphatic fluid instead of the portal blood. The lymphatic system provides a short-circuit bypassing the liver and draining its fluid directly into the systemic circulation via the thoracic duct.

Although restraint can be shown over what is ingested and it is possible to avoid eating until the surrounding area is safe and clean, there is little choice over what is inhaled. It usually has to be the air around us, unless a portable supply is to hand, and breathing cannot be suspended for more than 2 to 3 minutes without deleterious consequences. Assuming at rest, twelve breaths per minute and a tidal volume of 500ml, 8640 litres of air are taken into the lungs every day. The lungs have a large surface area, equivalent to that of a tennis court, and very delicate membranes which have evolved to act as gaseous exchange surfaces. Compounds, if volatile, can easily and rapidly pass into the blood stream from the lungs and this "portal of entry" initially circumvents the liver with its detoxication capacity.

The Toxicity Process

Once a compound has entered the systemic circulation it can be distributed around the body in a matter of minutes. Whether or not it actually enters a particular tissue depends upon a variety of factors, but some tissues may be particularly susceptible whereas others may have extra protection. For instance, the central nervous system is surrounded by layers of lipid and protein collectively called the "blood-brain barrier" which protects it from water-soluble ionic compounds but it is readily pemeable to many fat-soluble substances. In different circumstances, depending upon the toxicity of the compounds involved, this may be either beneficial or damaging.

Damage to a cell can occur in many different ways. These events may lead to the eventual death of the cell, and if sufficient cells are destroyed this will lead to the death of a tissue or an entire organ. Alternatively, they may cause a proliferative response where the cells may be damaged but nevertheless grow and divide causing organ enlargement or neoplasia. The sequence of events displayed by a cell showing a toxic response can be complicated. It is difficult to tease apart cause and effect, especially if an initial effect leads on to further responses. In this respect, observations of changes within a cell are usually classified under primary, secondary and tertiary stages, although there may be significant overlap.

Those processes included within the primary stage can be thought of as "event initiators" and involve the generation of highly reactive chemical entities such as free radicals or other electrophilic species and the decrease of free thiol levels within the cell, especially glutathione which acts as a vital protective agent. The loss or reduction of blood flow to a tissue depletes oxygen supplies which also aids cellular damage. Once produced, reactive chemical species, especially free radicals, can cause a cascade of peroxidative damage which leads to alteration in the structure and configuration of lipid components. Like a wave caused by a stone thrown into water, the initial chemical reaction is self-propagating, wreaking damage whilst liberating other free radicals to ripple outwards and continue the assault.

These events lead to a general macromolecular disruption which proceeds to secondary consequences. Both organelle and cell membranes, which are composed of a protein and phospholipid mosaic, can be damaged by these peroxidative changes. Alterations in fluidity and permeability lead to changes in intracellular ion concentrations, particularly calcium, and leakage of enzymes, both from intracellular organelles into the cytoplasm and from the entire cell into the intercellular medium. Interference with the correct functioning of mitochondrial membranes will eventually result in a shortage of high energy ATP, leading to a decrease in the ability to repair ongoing cellular damage. The rising levels of intracellular calcium interfere with cytoskeletal function leading to cellular disorganisation and also, perhaps more importantly, the activation of a series of autodestructive enzymes which degrade proteins and phospholipids and in particular the endonucleases which disassemble DNA in an ordered fashion during the process of apoptosis or "programmed cell death".

Finally, during the tertiary stage, gross changes to the cell's appearance occur. Steatosis, or the accumulation of fat, may take place owing to disruption in lipid handling. The intake of water, owing to membrane disfunction, causes swelling of the cell and is termed hydropic degeneration. The cell membrane may appear pitted or blebbed and vacuoles may form within the cytoplasm. Eventually, the endoplasmic reticulum and mitochondria may become grossly distorted, leak and rupture. The nucleus loses its structure, with the nuclear material condensing and becoming fragmented or just simply fading and dissolving away. These latter processes of irreversible damage are known as necrosis.

Provision of a Toxin

A pollutant or contaminant is usually regarded as that which makes something else impure by contact or mixture, to make it foul or filthy, to defile, sully, taint or infect. To pollute in common usage is generally taken as being "more dirty" than to contaminate. However, this is not always sharply defined. That which may be a contaminant or pollutant in one particular situation may not be so in another. For example, a red poppy in a garden is a desirable thing, a delicate and pretty flower adding its own beauty to the surroundings. A few perhaps out of place but growing wild in a hedgerow are also pleasant, but when the numbers increase vastly and poppies grow unrestricted in a corn field then they present a problem. Poppy seeds harvested with the adjacent corn will contaminate the grain and, if in sufficient concentration, will render it unusable. A contaminant, therefore, appears to be something "out of place" and usually, although not always, has to be in a high enough concentration to produce a problem. To add confusion, opinions also change as to what is a contaminant; some are obvious to recognise whereas others are not. Within a short period of fifteen years, five elements (selenium 1957, chromium 1959, tin 1970, vanadium 1971, fluorine 1972) which had been regarded previously as only environmental contaminants were shown to be beneficial micronutrients assisting in the continuation of life. This is only one example which has prompted concern to be expressed over the current continuing trend to "overpurify" the environment.

The population usually tries to live in areas which present the least problems in terms of immediate toxic hazard. However, certain communities do appear to dwell in locations which may be somewhat risky, perhaps unknowingly or because it is accepted as a part of their everyday life. Indeed, some individuals deliberately expose themselves to dangerous materials for a variety of reasons. Nevertheless, the surroundings in which we live and work are not devoid of toxic substances. Chemicals from industry and agriculture are present in all parts of the environment and may eventually accumulate in food chains. Direct exposure in our everyday work may also be a problem.

In general, a contaminant or potentially hazardous material may be presented to the population in one of several ways. It may be uncovered by natural processes such as water erosion or a volcanic eruption or it may be brought to the surface by man-made activities such as mining or quarrying. Alternatively, it may be a new problem in that it is a completely novel synthetic compound not encountered before in nature, or it may be a natural product which is now, owing to man's intervention, produced in quantities not previously seen.

When a potentially hazardous material is widely dispersed it is usually innocuous, and this is the ideology behind diluting a substance out of significance, although the logical extension of dumping waste materials into the vast oceans may not be too wise. For a "toxic event" or "toxic episode" to occur, areas of relatively high concentration of the hazardous material are usually required, although, of course, there are exceptions.

Such factors which bring about high local concentrations within a confined space can again be natural or man-made, or a combination of both. Rain soaking into the ground dissolves low concentrations of materials and then brings together that which has been leached out of perhaps thousands of square miles of land into a river valley, estuary and eventually a delta where the fast moving water hits the relatively static ocean and deposits the silt it has been carrying onto a fertile flood plain. A ideal location for a community to settle and thrive. If man has interfered and this hinterland is covered with mining spoil tips, composed of rejected material brought up from deep below the surface where it has been adequately hidden for aeons, then leaching of materials, especially heavy metals, will be enhanced and potential endemic problems exacerbated. Man-made structures, such as mines, quarries, factories and even cities can also serve to actively concentrate materials which are either extracted or artificially brought into these areas and then handled within confined spaces thereby delimiting their dispersion.

One interesting aspect is that of biomagnification. This is the process whereby a compound which appears in extremely low and harmless amounts, usually within the oceans, is concentrated as it passes through the food chain. Plankton within the seas may accumulate a toxin as they feed on passing particulate matter. Other sea creatures may then ingest this plankton and they themselves are eaten by a series of larger and more predacious creatures until relatively high concentrations reside within the fish population. Provided that the accumulated toxin is not poisonous, or has not yet reached a level where it is poisonous, to the fish, then this may be passed on to animals or humans who eat seafood. Eventually, when the concentration reaches toxic levels, or if a species is more susceptible than others, then damage, disease and possible death ensue. Concentrations in the order of a million-fold or more are not unusual within this process.

The following chapters give examples of substances derived from the surrounding biosphere. Complex organic molecules and simple inorganic moieties have been included, originating from the earth itself, constructed by the fascinatingly complicated intermediary biochemistry of living organisms or designed for nefarious reasons by the hand of man. Some of these may be familiar and others not, but all can be potentially lethal, justifiably earning them the appellation, "molecules of death".

Further Reading

Mason SF (1990). *Chemical Evolution*. Oxford University Press, Oxford.
Timbrell JA (2000). *Principles of Biochemical Toxicology,* 3rd ed. Taylor & Francis, London.