Part I:

A typical **intimal fibrofatty atherosclerotic plaque**, demonstrates a fibrocellular cap and a lipid core (containing macrophages and an extracellular lipid pool with cholesterol crystals). There is movement of circulating monocytes into the tunica intima to form tissue macrophages, where the macrophages phagocytose LDL and oxidised LDL to form foam cells. Smooth muscle cells migrate from the tunica media into the tunica intima, proliferate, change phenotype to secretory myofibroblasts, and secrete extracellular matrix including fibrous tissue. Atherosclerosis is primarily an intimal process affecting large-medium sized muscular arteries (e.g. coronary & carotid arteries) & large elastic arteries (e.g. aorta), although there may be thinning of the tunica media underneath a plaque.

**QA** At the earliest stages of plaque development, what influences lipid circulating in the blood, in the form of Low Density Lipoprotein (LDL), to enter the tunica intima of the artery wall?

**AA** The **response to injury hypothesis** (first developed in Cambridge) postulates repeated injury to endothelium caused by the four “major” risk factors: smoking, hypertension, diabetes, hyperlipidaemia (high LDL & low HDL). Endothelial dysfunction following injury, plays a crucial, initiating role in plaque formation. This allows lipids to pass more readily from the bloodstream into the tunica intima as a consequence of the combination of hyperlipidaemia and endothelial dysfunction/injury.

**QB** What stimulates blood monocytes to enter the arterial wall (becoming tissue macrophages) at the same site as the LDL?

**AB** Endothelial dysfunction/injury also triggers increased adherence of monocytes and platelets. Macrophages enter the arterial wall at this site. Both macrophages and platelets, when activated can release cytokines.

**QC** If the macrophages can phagocytose LDL forming “foam cells” (containing intracellular lipid), how does the extracellular lipid pool form and grow larger? (seen as cholesterol crystals and lipid gruel in your section).

**AC** Macrophages within the intimal plaque imbibe lipid to become “foam cells”. If the lipid remains intracellular at this early stage then only “fatty streaks or dots” are seen (usually in children/young adults). A causal relationship between atherosclerosis and fatty streaks/dots in children is suspected but not proved. Native and oxidised LDL can activate macrophages. Macrophage activation by the LDL, leads to free radical (ROS) production. The ROS oxidise LDL. However, oxidised LDL is toxic to macrophages inducing death by both necrosis and
apoptosis, causing release of lipid to form extracellular lipid pools with necrotic cell debris ("gruel"). Positive feedback occurs and there is a process with features of chronic inflammation in the vessel wall.

QD What triggers the smooth muscle cells in the tunica media to migrate to the tunica intima, proliferate and secrete extracellular matrix?

AD Macrophages become activated by oxidised LDL and secrete factors (as well as platelet release of PDGF) that encourage the recruitment of smooth muscle cells from the tunica media into the tunica intima, which proliferate and secrete collagen and extracellular matrix; macrophages also secrete enzymes & cytokines, etc that propagate injury.

QE Can you assign the four major risk factors (smoking, hypertension, diabetes mellitus & hyperlipidaemia) to pathogenic events in atherosclerosis?

AE All 4 “major” risk factors: smoking, hypertension, diabetes, hyperlipidaemia (high LDL & low HDL), can cause endothelial dysfunction and injury, the key initiating role in plaque formation. The “major” risk factors: diabetes & hyperlipidaemia can alter blood lipid levels, in particular by increasing LDL & reducing HDL levels. Entry of LDL (resulting from high circulating levels of LDL) into the arterial wall at a site of endothelial dysfunction / injury is the important early event in atherosclerotic plaque formation.

QF What makes an atherosclerotic plaque rupture? Why is this an important event?

AF Rupture or ulceration of a plaque, by definition, implies loss of the overlying endothelium. Rupture of an atheromatous plaque is usually associated with a breach of the fibrocellular cap and, hence, exposure of connective tissue collagen and the lipid core to the bloodstream. The latter is important as it exposes additional thrombogenic substances to the blood and also allows for discharge of debris (release of cholesterol emboli) into the bloodstream. Plaque rupture is probably caused by a combination of several factors. The overlying endothelium and fibrocellular cap may be mechanically weakened externally by turbulent blood flow (generated by partial occlusion of the blood vessel by neighbouring plaques and the plaque itself) and/or internally by a haematoma (from e.g. the thin walled capillaries vascularising the plaque) growing within the plaque. Calcification of the fibrocellular cap reduces its mechanical compliance and further predisposes to rupture. It is suggested that proteolytic enzymes released by ‘intraplaque’ macrophages and other leucocytes may disrupt and weaken the fibrocellular cap. Finally, while endothelial injury may be the initiating step of atherosclerosis, exposure to oxidised LDLS, cytokines and proteolytic enzymes generated within the plaque may further damage and disrupt the overlying endothelium and cap.

This is an important event because plaque rupture leads to thrombosis! Rupture of a plaque exposes subendothelial tissue and the lipid core and hence, as mentioned above, thrombogenic substances to the bloodstream (eg. von Willebrand’s factor, tissue factor, collagen). Thrombosis of a coronary or carotid arterial plaque has obvious potentially fatal consequences!
**Part II:**

Q1 Work out, and justify as fully as possible, whether P22.5 was taken earlier than P22.6 or the other way round.

A1 In P22.5, the myocytes appear to have lost their characteristic striations. Many are eosinophilic, due to coagulative necrosis of the proteins within them, increasing the uptake of eosin. The nuclei exhibit karyolysis (diminished staining/fading of the nuclei) and karyorrhexis (nuclear disintegration). Some nuclei have already disappeared. Infiltration with neutrophils is beginning to occur, but no macrophages or signs of repair can be seen. Therefore this photograph shows the microscopic appearance of the myocardium 1-2 days after a myocardial infarction. P22.6, on the other hand, shows granulation tissue between the remaining myocytes. Granulation tissue consists of dilated thin walled blood vessels, fibroblasts beginning to lay down collagen, and macrophages phagocytosing the remaining cellular debris. Therefore, P22.6 shows the myocardial appearance 7 - 10 days after a myocardial infarction.

Q2 What changes in the myocardial cells can explain (i) the raised levels of Troponin-I and MB isotype of creatine kinase; (ii) the pain?

A2 (i) Myocardial cells must liberate intracellular contents, if the blood levels of Troponin-I and CK-MB are to rise. Therefore the cells in the infarct have undergone necrosis, with breakdown of the plasma membranes and release of intracellular molecules including Troponin-I and CK-MB proteins. (ii) Pain usually results from the detection of the products of anaerobic metabolism, by nerve fibres within the myocardium in the zone of ischaemia around the infarct. Few structural changes will be seen in this zone, apart from some sublethal hydropic change in a few severely ischaemic cells. Dead areas of myocardium (with dead non-functioning nerves) will not cause pain.

Q3 What might you have found in a microscope section of the coronary artery, which supplied the infarcted wall of the left ventricle?

A3 The artery is most likely to be obstructed by a recent thrombus forming on a ruptured atherosclerotic plaque (rupture of the overlying fibrous cap, with exposure of the extracellular pool of lipid in the core of the plaque and the surrounding extracellular fibrous matrix synthesised by the smooth muscle cells that have migrated into the intima).

Q4 What changes to the intestines can you see? How has this occurred? Why would this cause death?

A4 P22.7 A large continuous dusky blue-black area can be seen in the intestine. This is because an embolic infarct has occurred. The thrombo-embolus has arisen from left ventricular mural thrombus, due to the underlying recent myocardial infarction. Death results from septicaemia (due to bacteria in the blood), as the dead intestine no longer provides a bacterium-proof layer, thus allowing gut bacteria to enter the blood stream. Bacteria in the blood cause inflammation with vasodilatation leading to septic shock and death.

Q5 What has occurred to the brain and why did it occur?
A5 **P22.8** An infarct can be seen in this post mortem brain. This has caused a large area of tissue to look pale and rather gelatinous. This also resulted from thrombo-embolus from left ventricular mural thrombus.

The right-sided paralysis is due to injury and death of motor neurones in the brain. The cause of this brain infarct is embolism of **thrombus within the heart chambers**. Thrombus has formed over the area of infarction of cardiac muscle. This thrombus has then embolised to the brain to cause infarction of the motor cortex in the brain.

Another possible cause of brain infarction might be carotid artery atherosclerosis. Since atherosclerosis affects arteries in many parts of the body, a second focus might have precipitated a thrombotic episode involving the arterial supply (e.g. Carotid Artery or Middle Cerebral Artery) to the motor cortex in the brain.

In this case it is clear that there are multiple embolic events involving other organs as well - presumably from a single source, so a mural thrombus overlying a myocardial infarct is much more likely to be the cause.

Q6 An abnormality was found in his kidney at post mortem. What shape is it? What does this suggest it is? Correlate the naked eye changes with the histological appearances.

A6 **P22.9 & P22.10** Within this kidney, a wedge shaped pale area can be seen. This is also an infarct resulting from thrombo-embolus from the left ventricular mural thrombus to a branch of the renal artery. Histology confirms this. A well demarcated area can be seen in which the cells appear ghostly, often with absent nuclei, although the architecture of the tissue is still discernible – the typical features of necrosis. Neutrophil infiltration at the edges of the infarct can also be seen.

Q7 Can you draw a flow diagram summarising the sequence of events from the pathological processes that triggered the myocardial infarction, to changes affecting brain, kidney & intestines, eventually leading to death?

**Flow chart:**

Coronary artery atherosclerosis → plaque rupture → thrombus formation → occlusion of the artery → myocardial infarction → left ventricular mural thrombus → multiple emboli to a number of organs including brain (infarction with hemiplegia), kidney (infarction), and intestines (infarction resulting in movement of bacteria across the necrotic intestine wall into the blood stream → bacteraemia (small numbers of bacteria in the blood) → septicaemia (large numbers of proliferating bacteria in the blood) → septic shock (shock: increased heart rate + decreased blood pressure as a consequence of bacteria-induced inflammatory processes being activated throughout the circulation causing widespread vasodilatation) → death.