Write your name here

Surname	Other names

Pearson Edexcel
International
Advanced Level

Centre Number | | | | | |

Candidate Number | | | | |

Biology

Advanced

Unit 5: Energy, Exercise and Coordination

Thursday 15 January 2015 – Morning
Time: 1 hour 45 minutes

Paper Reference
WBI05/01

You must have:
A copy of the scientific article (enclosed), calculator

Total Marks

Instructions

- Use **black** ink or ball-point pen.
- **Fill in the boxes** at the top of this page with your name, centre number and candidate number.
- Answer **all** questions.
- Answer the questions in the spaces provided
  – *there may be more space than you need.*

Information

- The total mark for this paper is 90.
- The marks for **each** question are shown in brackets
  – *use this as a guide as to how much time to spend on each question.*
- Questions labelled with an **asterisk** (*) are ones where the quality of your written communication will be assessed
  – *you should take particular care with your spelling, punctuation and grammar, as well as the clarity of expression, on these questions.*
- Candidates may use a calculator.

Advice

- Read each question carefully before you start to answer it.
- Keep an eye on the time.
- Try to answer every question.
- Check your answers if you have time at the end.

Turn over ▶

Answer ALL questions.

Some questions must be answered with a cross in a box ☒. If you change your mind about an answer, put a line through the box ☒ and then mark your new answer with a cross ☒.

1 Organisms need to coordinate responses to changes in their environment.
The mechanism of coordination in animals can be nervous or hormonal.

(a) Place a cross ☒ in the box next to the answer that correctly compares nervous coordination with hormonal coordination.

(1)

☒ **A** nervous coordination is faster and lasts for a longer time

☒ **B** nervous coordination is faster and lasts for a shorter time

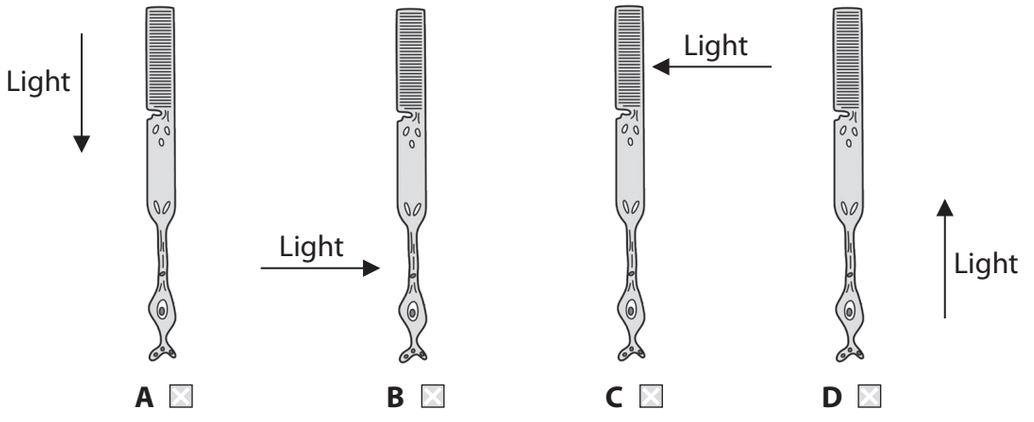
☒ **C** nervous coordination is slower and lasts for a longer time

☒ **D** nervous coordination is slower and lasts for a shorter time

(b) The response to light in humans involves rod cells as receptors.

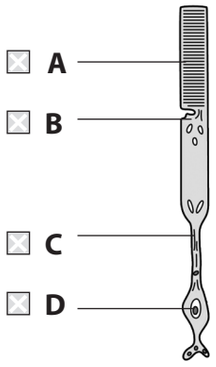
(i) Place a cross ☒ in the box below the diagram that shows the direction light takes when it stimulates a rod cell.

(1)



(ii) Place a cross ☒ in the box next to the part of the rod cell that contains rhodopsin.

(1)



(iii) Place a cross ☒ in the box next to the description of what happens when a molecule of rhodopsin is bleached by light.

(1)

☒ **A** opsin changes to retinal

☒ **B** retinal changes to opsin

☒ **C** trans-retinal changes to cis-retinal

☒ **D** cis-retinal changes to trans-retinal

(iv) Bleaching of rhodopsin leads to hyperpolarisation of the rod cell membrane. Place a cross ☒ in the box next to the description of what happens during hyperpolarisation.

(1)

☒ **A** sodium ion channels close while the sodium ion pump stops working

☒ **B** sodium ion channels close while the sodium ion pump continues to work

☒ **C** sodium ion channels open while the sodium ion pump continues to work

☒ **D** sodium ion channels open while the sodium ion pump stops working

(c) Coordination in plants involves IAA (auxin).

In an experiment, 25 mm lengths of stem were cut and placed in five dishes. A different concentration of IAA was added to each dish. The dishes were left for 24 hours and the mean increase in stem length was recorded.

The results are shown in the table below.

Dish	IAA concentration / mg dm^{-3}	Mean increase in stem length / mm
1	0.00	2.5 ± 1.0
2	0.01	2.1 ± 1.5
3	0.10	5.0 ± 1.1
4	1.00	6.8 ± 4.0
5	10.00	7.8 ± 3.2

(i) Use the information in the table to describe the effect of IAA concentration on the mean increase in stem length.

(2)

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(ii) Suggest **one** other variable that needs to be controlled in this experiment.

(1)

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(iii) It is important that the calculated means are reliable.

Using the information in the table, state the mean result that is the **least** reliable. Give a reason for your answer.

(1)

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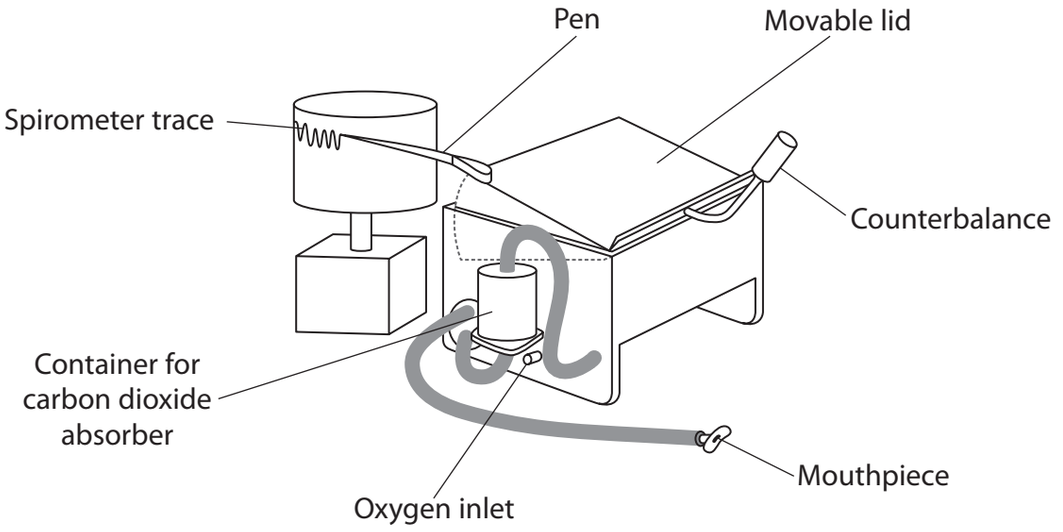
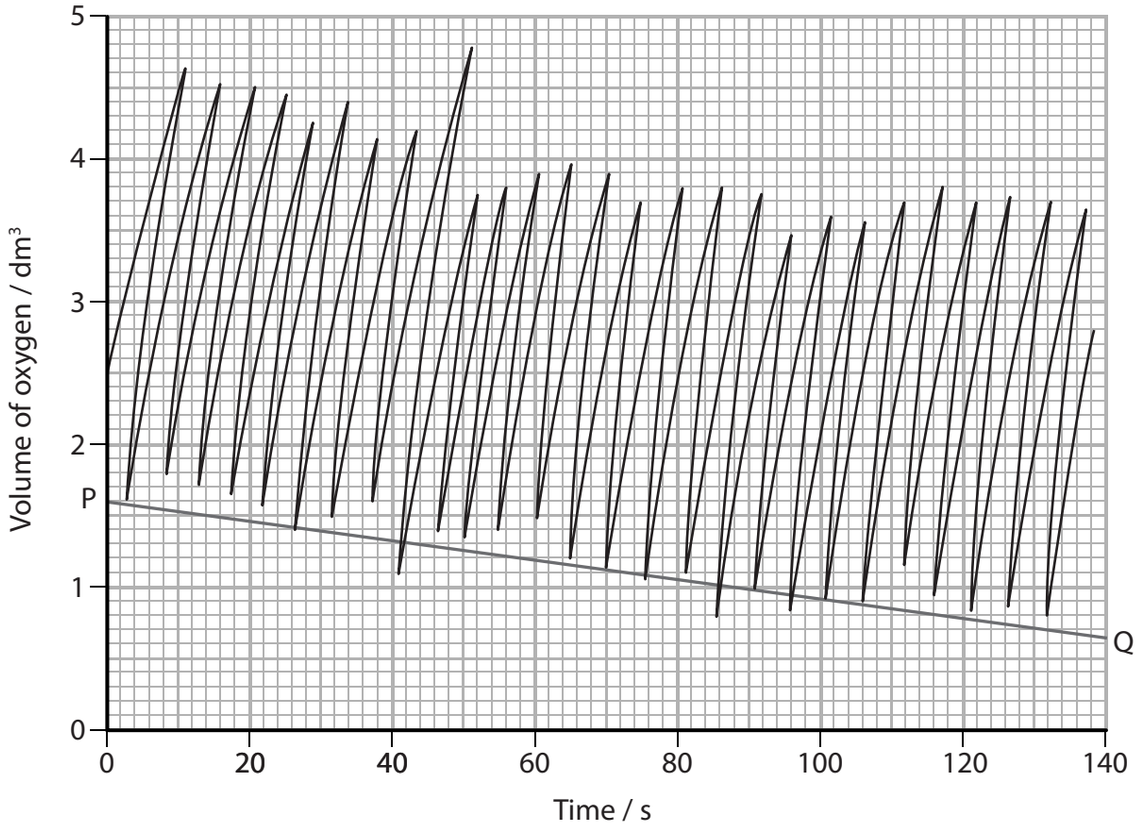
(Total for Question 1 = 9 marks)

2 The diagram below shows a spirometer that can be used to measure lung volumes. A spirometer can also be used to measure the volume of oxygen a person uses.



(a) A student used a spirometer to measure the volume of oxygen he used at rest and during exercise.

The spirometer trace below shows the results he obtained during exercise.



(i) The line P to Q slopes downwards because oxygen is being used.

Use the line, labelled P to Q on the trace, to calculate the volume of oxygen used during one minute of exercise.

(1)

Volume of oxygen used = ...

(ii) The student had a body mass of 70 kg.
Calculate the rate of oxygen used by this student in $dm^3\ kg^{-1}\ h^{-1}$.

Show your working.

(2)

Rate of oxygen used = ... $dm^3\ kg^{-1}\ h^{-1}$

(iii) Suggest **two** differences between this spirometer trace and the one the student obtained at rest.

(2)

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(b) (i) The air the student exhaled passed through the carbon dioxide absorber in the spirometer.

Name a carbon dioxide absorber.

(1)

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(ii) Explain why the spirometer trace would be different if the carbon dioxide had not been absorbed.

(2)

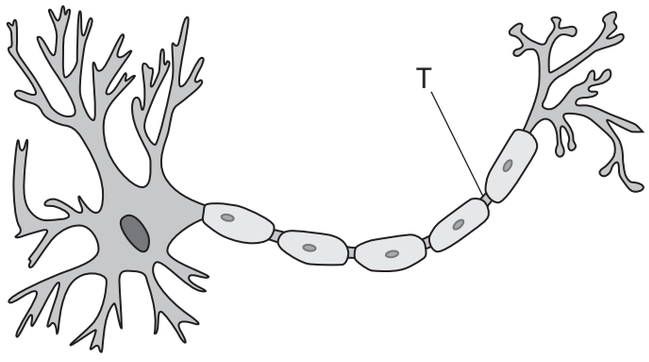
(c) Explain how carbon dioxide is involved in the control of breathing rate during exercise.

(4)

(Total for Question 2 = 12 marks)

3 Nerve impulses are transmitted along the axon of a neurone.

(a) The diagram below shows the structure of a motor neurone.



(i) Place a cross ☒ in the box next to the part of the neurone labelled T.

(1)

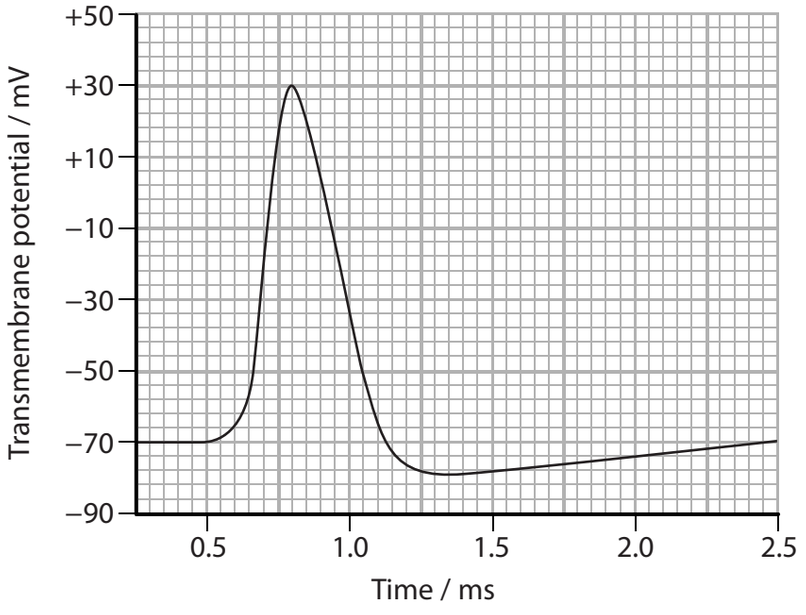
☒ **A** dendrite

☒ **B** node of Ranvier

☒ **C** Schwann cell

☒ **D** synapse

(ii) The graph below shows changes in the membrane potential during the transmission of an impulse along the axon of a motor neurone.



Place a cross ☒ in the box next to the description of the membrane potential at 0.75 ms on the graph.

(1)

☒ **A** depolarised

☒ **B** hyperpolarised

☒ **C** polarised

☒ **D** repolarised

(iii) Explain how the structure of this motor neurone affects the speed of the impulse along the axon.

(2)

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(b) The photograph shows a golden poison frog (*Phyllobates terribilis*).

Magnification ×1

The skin of this frog produces a poison that affects sodium ion channels in the axon membrane of a neurone. The poison causes these channels to stay open.

(i) Explain the effect the poison has on the ability of a neurone to transmit impulses.

(4)

(ii) Suggest why the neurones of the golden poison frog are not affected if they come into contact with the poison.

(2)

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(Total for Question 3 = 10 marks)

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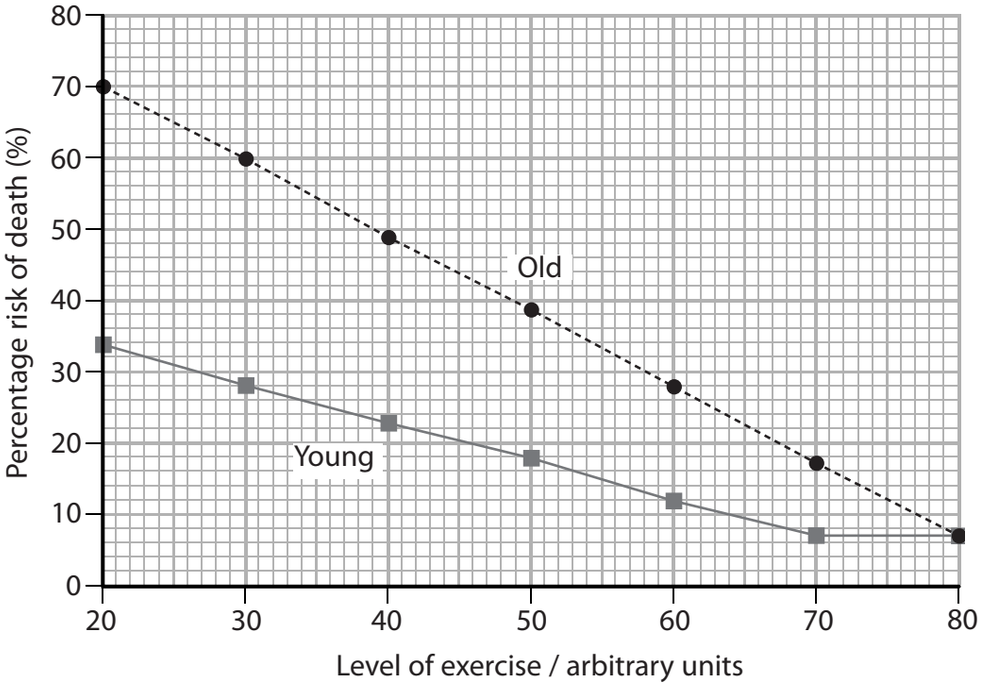
4 Exercise is important for human health.

The graph below shows how level of exercise changes the risk of death in young and old people.



(a) Use the information in the graph to describe the effect of level of exercise on the percentage risk of death.

(3)

(b) State the effect of exercise on each of the following.

(i) The risk of having diabetes and being obese.

(1)

(ii) The immune system.

(1)

(c) Exercise has an effect on the risk of having coronary heart disease (CHD).
In people with CHD the heart muscle cells receive less oxygen.

*(i) Describe how heart muscle cells make ATP when less oxygen is available.

(6)

(ii) A person will suffer a pain called angina if heart muscle cells receive less oxygen.

Suggest how lack of oxygen in heart muscle cells can cause angina.

(2)

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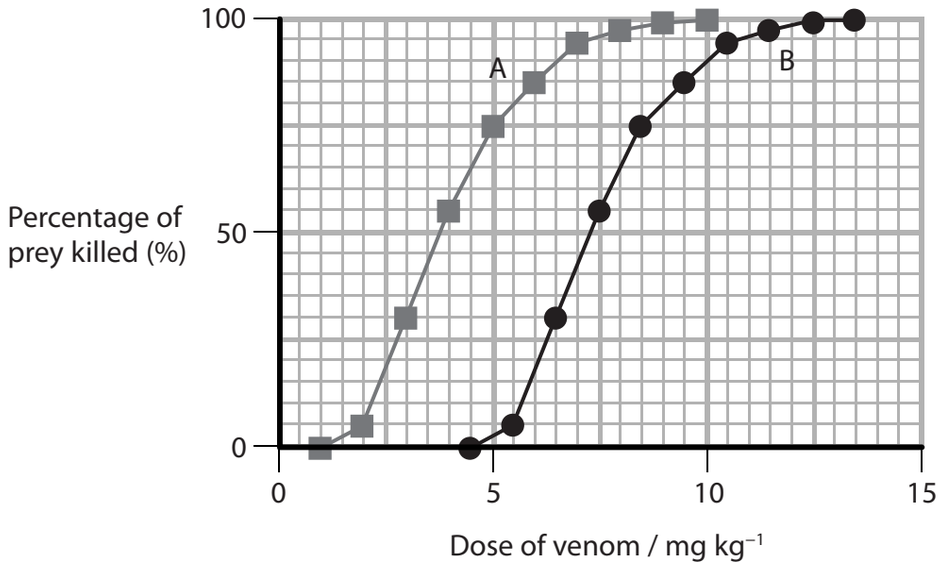
(Total for Question 4 = 13 marks)

5 Some animals, such as spiders, bite and inject venom into their prey. The venom affects the transmission of nerve impulses and paralyses the prey.

In an investigation, scientists estimated the toxicity of the venom by measuring the dose that can kill 50% of prey after injection. This dose is called the LD_{50}.

The graph below shows the relationship between the percentage of prey killed and the dose of venom A and venom B.



(a) (i) Use the information in the graph to compare the toxicity of venom A and venom B.

(2)

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(ii) Place a cross ☒ in the box next to the independent variable in this investigation.

☒ **A** dose of venom

☒ **B** mass of the prey

☒ **C** percentage of prey killed

☒ **D** toxicity of the venom

(1)

(b) Two scientists, P and Q, investigated the LD_{50} values for the venom produced by four species of spider. Their results are shown in the table below.

Scientist	Species of spider	LD_{50}
P	*L. mactans*	0.002 mg kg^{-1}
	H. huwenum	0.700 mg kg^{-1}
Q	*P. bahiensis*	0.610 μg kg^{-1}
	A. robustus	160.0 μg kg^{-1}

Scientist P measured the toxicity in mg kg^{-1} and scientist Q measured the toxicity in μg kg^{-1}. One μg is equal to 0.001 mg.

Name the species with the most toxic venom.

(1)

.....

(c) The venom from *L. mactans* (black widow spider) causes constant impulses to be sent along motor neurones. This results in cramps (constant muscle contractions), a symptom of a black widow spider bite.

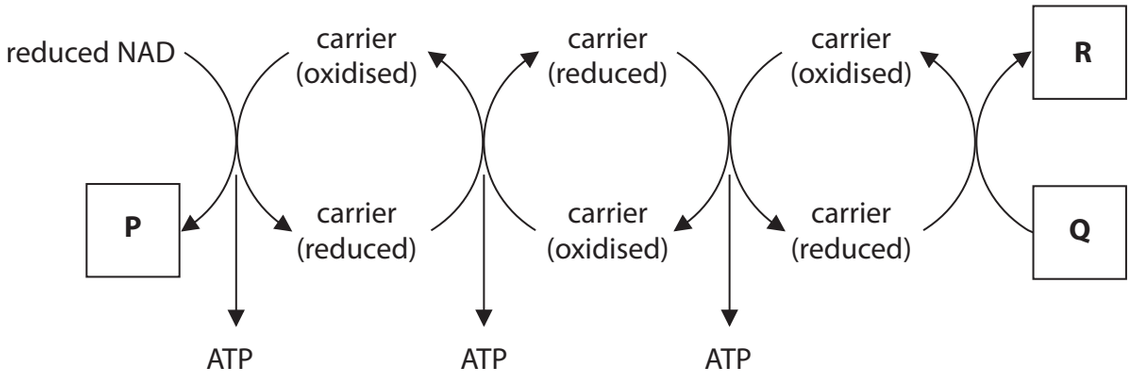
Suggest how constant impulses along motor neurones cause cramps.

(4)

(Total for Question 5 = 8 marks)

6 The electron transport chain is involved in the synthesis of ATP.

(a) The diagram below shows part of the electron transport chain.



(i) Name the molecules P, Q and R.

(3)

P .....

Q .....

R .....

(ii) Place a cross ☒ in the box next to the description of where the electron transport chain occurs.

(1)

☒ **A** cytoplasm surrounding mitochondria

☒ **B** inner mitochondrial membrane

☒ **C** mitochondrial matrix

☒ **D** outer mitochondrial membrane

(b) In 2013, poachers killed over 80 elephants in Zimbabwe by poisoning their drinking water with cyanide. Cyanide inhibits cytochrome oxidase, the last carrier in the electron transport chain.

Suggest how inhibiting cytochrome oxidase would kill an elephant.

(4)

(Total for Question 6 = 8 marks)

7 The scientific article you have studied has been adapted from a Royal Society publication. Use the information in the article and your own knowledge to answer the following questions.

(a) Scientists are encouraged to use non-animal alternatives in their research (paragraphs 3 and 5).

Name **one** non-animal alternative that can be used in research and give an advantage of this alternative method.

(2)

(b) Explain why new medicines are tested on animals before they are tested on humans (paragraph 7).

(2)

(c) The article states that "Molecular mechanisms and those involved in cell differentiation and propagation are frequently identical across a wide range of species" (paragraph 11).

Discuss this statement by making reference to the molecular mechanisms involved in switching genes on and off.

(4)

(d) Suggest the advantages of using mice as a "knock out" model in research compared with the use of humans (paragraph 12).

(2)

(e) Suggest how the experimental design for the testing of thalidomide could have been improved (paragraph 13).

(1)

(f) State what is meant by the term **mouse genome** (paragraph 15).

(1)

(g) Explain why animals had to be used to test the polio vaccine (paragraphs 17 and 18).

(2)

(h) The article states that drug-resistant infections "are a major problem in both developed and developing countries" (paragraph 22).

Explain how drug-resistance evolves in organisms that cause infection.

(3)

(i) The article discusses absolutism as an ethical position with regard to animal testing (paragraphs 28 and 29).

Suggest what is meant by the term **absolutism**.

(2)

(j) The article states that there is an "alternative to such absolutism" (paragraph 29). People who believe in this alternative ethical position are called relativists.

Suggest the opinion a relativist would have with regard to using animals in research.

(1)

(k) The article states that scientists who use animals for testing are expected "to reduce the number of animals used in research to the minimum required for meaningful results" (paragraph 37).

Explain why reducing numbers below the minimum required could produce results that are not meaningful.

(2)

(l) Suggest how gene therapy for "motor neuron degeneration diseases" such as ALS might be carried out (paragraph 42).

(3)

*(m) An investigation that used animals was carried out by Hubel and Weisel. These scientists used kittens to investigate brain development.

Explain how this work helped to develop explanations of human brain development.

(5)

(Total for Question 7 = 30 marks)

TOTAL FOR PAPER = 90 MARKS

8. Within this article, the following issues are considered: examples of medical advances that have been achieved through the use of animals; the theoretical framework behind the use of animals; the legislation that regulates the use of animals; and discussion of philosophies that underpin the debate about the use of animals in research. This article is not intended to be a manual of how to conduct research. Nor is it a substitute for good advice on experimental design, statistical analysis or any of the other aspects of conducting a successful research project.

9. The understanding of the human body has come from more than 200 years of research on the function of normal cells, tissues and organs, and on disease processes. Much of this understanding has been facilitated by research that was performed on animals. This fundamental knowledge underpins the teaching of medicine and veterinary medicine and has been instrumental in the development of medical advances for both people and animals.

10. It is no exaggeration to say that almost every form of conventional medical treatment, such as drugs, vaccines, radiation or surgery, rests in part on the study of animals. However, this fundamental link is frequently not appreciated and the following examples illustrate some of the important medical advances that have resulted directly from animal experiments.

11. Model species are used to test possibilities that would be difficult or impossible to test using the target species. In general, one species may be used as a model for another when, despite other differences between them, the two species strongly resemble each other in particular ways. Molecular mechanisms and those involved in cell differentiation and propagation are frequently identical across a wide range of species. Therefore, for some of the most fundamental principles of biology, using animals as models can provide valuable insight into human cell processes.

12. Animal models also enable a much greater control of experimental conditions than could reasonably be achieved in humans. Human patients can be highly heterogenous in disease symptoms and their behavioural variability, such as differences in patients' compliance with instructions, is far more significant in human trials. In certain uses of animals as models, such as mouse 'knock-out' models, in which individual genes are switched off to study the effect, researchers can even control the genetic make-up of the experimental subjects to ensure homogeneity.

13. Opponents to the use of animals in research claim that using animals as models for humans is invalidated by the differences between humans and animals. Evidence presented to support this argument includes the case of the unpredicted limb defects that occurred in the children of women who took the drug thalidomide during pregnancy. It is true that the damaging effects of thalidomide on developing embryos were not predicted in the initial animal experiments but this is because the effects of thalidomide during pregnancy were not looked for. Had the researchers at the time attempted to anticipate these effects, then research conducted using pregnant animal research subjects would have been able to detect these effects, as seen in later studies conducted after the effects of thalidomide in humans were discovered and the drug was withdrawn.

14. It is important to emphasise that animals are normally highly accurate models for humans, and in this instance it was experimental design that failed to look for the teratogenic effects, not that the experiments failed to detect the effects. The similarity between some animals and humans is best demonstrated by the fact that many drugs can be used to treat both human and animal patients, such as antibiotics and tranquillisers.

15. The last two decades have witnessed a revolution in biology with the sequencing of the human, mouse and fly genomes. A wealth of information has been produced about the role of genes and gene products in some diseases, as well as providing insight on why some people respond to some medicines better than others. The knowledge now available from the sequencing of the human, mouse and fly genomes has enabled genetic modification of animals to produce highly specific models of diseases, helping to identify disease pathways and aid the development of new therapies.

16. Following are three case studies that provide examples of medical advances that have been developed through the use of animals in research.

Case study on polio vaccine

17. An example of the use of animals as models of human cell processes is the development of polio vaccine. Polio is an infectious disease that can strike at any age but mainly affects children under three years old, and is prevalent in developing countries. The polio virus enters through the mouth and once it enters the blood stream can invade the central nervous system, destroying nerve cells in the limbs, trunk and the brainstem, resulting in paralysis and sometimes death. Soon after the introduction of effective vaccines in the late 1950s and early 1960s, polio was brought under control, and practically eliminated as a public health problem in industrialized countries. Research into polio vaccine requires the use of living nerve tissue to ensure that the virus used for vaccine production causes the paralysis typical of polio, and no human or tissue culture alternative is available.

18. Additionally the polio vaccine uses a live attenuated virus, which is notorious for sometimes reverting to virulence, so animals are still the only practical way of predicting the potential virulence of each batch of polio vaccine. As a result of this potential risk to humans, each batch of vaccine is tested in animals. This previously involved an intra-cerebral injection of the vaccine into monkeys, which is highly predictive of virulence.

19. More recently mice have been genetically engineered to have the receptors for the virus, providing animal models of the disease. Despite the many differences between mice and humans, the use of genetically modified (GM) mice to establish the virulence of the vaccine provides an accurate model of humans in this respect. This is a good illustration of how mice, and in particular GM mice, can be used as models for human pathogens. Besides being genetically similar to humans, mice are small and inexpensive to maintain. Their short life span and rapid reproductive rate make it possible to study disease processes in many individuals, thus gaining a greater understanding of the progression of the disease within a short space of time.

Drugs for the future

20. A great number of diseases remain for which drug therapies are far from optimal or even nonexistent. Many drug targets are proteins and the completion of the human genome project, the blueprint for around 30 000 human genes and 250 000 proteins, has enabled the genes encoding for these proteins to become known and provides potential for a greater understanding of disease at the molecular level than at any other point in history.

21. Techniques such as genomics and proteomics can help provide a wealth of information about these proteins such as their functional role, and can help refine experimental procedures, but they cannot substitute for the complexity provided by whole subject studies for increasing understanding and developing treatments of disease. The long and complex route from discovery to drug will inevitably require the use of animals at some stage of the scientific investigation and it is important to realise the limitations of non-animal techniques.

22. Many challenges to the healthcare systems of both developed and developing countries exist that would benefit from the use of animals in research. Examples include: drug-resistant infections, which are a major problem in both developed and developing countries, frequently involve examination of disease progression and research could benefit from the use of animal models such as genetically modified mice; mental illnesses, especially depression, schizophrenia and anxiety, which require a holistic approach, involving molecular, cellular and whole animal studies; better treatments for diseases of bone, joints and the immune system, all of which involve complex interactions throughout the body; treatment of diseases of genetic origin, such as Duchenne muscular dystrophy, which could utilise genetically modified animals to mimic human patients; blindness and deafness, which involve analysis of these functions in living research subjects; HIV infection and AIDS, with animal models of the disease required to develop and test possible vaccines or antiviral agents before trials can be safely conducted in humans; senile dementia and other complications of old age, where the cause and progression of these conditions is poorly understood at present, and require detailed examination of the brain and nervous system in animal models.

Case study on cystic fibrosis

23. Cystic fibrosis is an example of a disease where future therapies are being developed through the use of genomics. Much research is focused on correcting the genetic deficiencies that lead to the disease either by gene therapy, which would turn the faulty protein into a functional protein, or by finding alternate pathways to bypass the need for the protein. The cystic fibrosis gene, cystic fibrosis transmembrane-conductance regulator or CFTR, codes for a large protein, CFTR protein, that functions as an epithelial chloride channel and has a regulatory influence on many other cellular proteins. Without a functional form of this protein a person suffers from a lethal genetic disease, cystic fibrosis.

24. In other conditions, such as the secretory diarrhoeas associated with cholera, CFTR is a key player in the devastating loss of bodily fluid. The need to discover high affinity ligands that interact with both CFTR protein and some of the common faulty forms of this protein is key to the development of cystic fibrosis therapies. A recent search for CFTR ligands was carried out using cells in culture expressing CFTR protein and a halide indicator, as normal CFTR functions as a chloride channel. In a recent search for agents that activated or inhibited the channel, CFTR protein was expressed in cultured cells that also contained a reporter molecule that altered its fluorescence when either chloride (or other halide) entered or left the cells.

25. By screening 50,000 diverse compounds, six compounds were discovered that blocked the activity of CFTR protein and further chemical modification led to several channel blockers with sub-micromolar activity. Having identified these six lead compounds using non-animal methods, further research was then carried out by testing in mice to establish the most active compound. What this example shows is the multiple methodologies involved in testing a potential therapy, and that the use of animals remains a key stage. The search for CFTR activators continues, which may prove useful for future therapies for cystic fibrosis, and further investigations will undoubtedly require the use of transgenic animals bearing common mutations of the CFTR gene.

Ethical approaches to the use of animals

26. All those involved in the debate about the use of animals in research lay claim to one or other moral principle. In human and veterinary medicine, causing pain or suffering in a patient is considered unethical unless it is for the direct benefit of that patient. Those who favour work on animals may do so to alleviate the suffering of humans or other animals. Scientists in favour of this principle use their research to understand fundamental aspects of biology that in turn facilitate the development of therapeutic measures for both animals and humans. Those who oppose the use of animals in research may object to the means by which scientists attempt to achieve their goals.

27. One view is that each animal has the right to life and humans should not take such a right away from it. It is not entirely clear whether the proponents of such a view would grant rights to every organism that showed signs of reacting to maltreatment. Nevertheless, they would argue that rights to good treatment, once granted, must be respected.

28. Others would argue that while granting rights to animals is inappropriate because human rights are firmly embedded in a social context, humans have responsibilities for animals in their care and should ensure that their welfare is good. Both the rights and the responsibilities arguments are sometimes taken as absolutes, over-riding all other moral claims. However, this could also be the case for the moral argument for supporting animal experiments because of their potential medical benefit.

29. The alternative to such absolutism is to respect the range of views by attempting to both minimise the suffering inflicted on animals used in research while maximising the scientific and medical gain. This is the position enshrined in UK law governing the use of animals in research. Balancing between these positions required by law is not an exact process since the assessment of scientific and medical benefit and that of animal suffering are both difficult to quantify and are not expressed in the same terms.

30. The assessments are incommensurate and, therefore, referring to the judgement as cost-benefit analysis is misleading. So the degree of suffering might be expressed as low, medium or high and the likely scientific and medical benefit might be similarly classified. Research that involves low suffering to the animals and was likely to be highly beneficial would generally be regarded as acceptable. Research that involves medium suffering but only a medium chance of generating a beneficial outcome would probably be deemed unacceptable – but clearly this judgement will depend on a consensus view derived from a judgement by those bodies responsible for granting approval to research projects.

31. Criticism of the use of animals in research sometimes arises when there appears to be no immediate tangible health benefit of the research. An inability to quantify the benefits of a research project can be seen to imply that it is frivolous or wasteful and therefore unethical. This is an invalid assumption, however, as research studies that do not have direct benefit to humans or other animals can instead provide a vital contribution to fundamental scientific understanding that may provide benefit in the future.

32. Individual experiments are similar to the individual bricks in a building, with knowledge being built up over a long period of time and with the benefits perhaps only being realised when the building or the research is completed. On the other hand, the benefits of applied research may be easier to quantify. For example, in drug development, many thousands of compounds may need to be tested in order to develop a new drug. This means that in some cases the research may not be successful, and may seem futile, whereas in fact such work is essential in refining knowledge.

33. It is therefore important when considering the ethical justification of the use of animals in research to realise that the development of a successful drug such as insulin or the antibiotics may result in saving many millions of human and animal lives.

Ethical issues surrounding the use of genetically modified animals

34. The potential benefits of using genetically modified animals for research are great, and there is a strong scientific case for using such animals in order to understand human and animal disease. However, the use of genetically modified animals in scientific research may raise additional ethical issues, such as concerns about possible additional welfare costs that may arise from the use of this technology, and ethical questions about using animals in this way.

35. A largely hypothetical concern with respect to genetic modification is that introducing a gene into an organism from a very different type of organism may lead to unforeseen interactions in development, leading to the emergence of animals that have serious welfare problems. However the targeted genetic modification approach used to create genetically modified animals for research purposes is likely to be more predictable than other methods of inducing genomic changes, such as radiation exposure or chemical mutagens.

36. Intensive behavioural studies have been conducted on GM animals to monitor any adverse welfare implications and these have found very little difference between animals that have been genetically modified and those that have not. Moreover, we should also bear in mind that animal breeders have been selecting for genetically determined traits for many centuries, such as the range of dog breeds, which are widely accepted but raise similar ethical and welfare issues to genetically modified animals.

Refinement, reduction and replacement in animal research

37. The guiding principles of animal welfare are the so-called 'three Rs', refinement, reduction and replacement, first clearly defined in 1959. This means that every effort must be made:

- to **refine** the procedures so that the degree of suffering is kept to a minimum.
- to **reduce** the number of animals used in research to the minimum required for meaningful results.
- to **replace** the use of live animals by non-animal alternatives.

38. Current UK legislation requires all researchers who propose to undertake laboratory or fieldwork involving animals to give full consideration to the three Rs and to seek independent advice and approval from a local ethics committee. The approvals process for personal and project licences emphasises that researchers should seek, where possible, to avoid the use of animals and must advance sound and detailed scientific arguments for their use, explaining why no realistic alternative exists. Scientists must also address what is the acceptable trade off between the welfare benefits of the three Rs approach and the costs of not obtaining a more definite result through using intact animals.

39. The principle of the three Rs is not to remove the use of animals in research, but instead to provide a mechanism to ensure the best possible use of animals in research. Using animals in scientific research provides a bedrock for increasing understanding, and is consistent with the three Rs as developing our knowledge helps to enhance existing techniques using animals, as well as generating knowledge that underpins research studies using humans.

Refinement

40. Refinement is defined as 'reducing to a minimum the incidence or severity of suffering experienced by those animals which have to be used'. Refinement of experimental methods, for instance through adequate post-operative care, good housing, and improved anaesthesia and analgesia has been standard practice in biomedical research for many years. Refinement of surgical procedures is frequently by technical advances but also by experimenters asking themselves some generic questions that can be asked prior to undertaking any invasive procedures: can I minimise the area of tissue at risk of damage or infection? Can any aspect of the surgical process and subsequent recovery be made less troubling to the animal?

Case study on refinement

41. A common question directed to researchers using animals is how can one be sure that the species being used is appropriate to the study of human health or illness. Selecting the appropriate model is an important step scientifically of course, but also in terms of animal welfare. The more one can predict the course of the disease induced in the animal, the better one can anticipate the animal's behaviours and needs such as changes in housing, diet or diurnal routine. Use of a certain species for research purposes is dependent on the process or disease under study. If one compares the genomes of the mouse and humans, one finds that there is a mouse homologue for 99% of human genes, and of the genes implicated in human disease processes, 90% are present in the mouse. As a consequence of this degree of similarity, major advances have been made recently by the development of transgenic mouse models of human neurodegenerative disease.

42. The identification of mutations in the SOD1 gene as the cause of Amyotrophic Lateral Sclerosis (ALS), a lethal neurodegenerative disease, has opened new possibilities. Mice with mutations in the SOD1 gene develop severe motor neuron degeneration similar to that seen in humans and targeting specific mutations in SOD1 has given researchers the ability to study specific symptoms such as limb weakness, axonal swelling and the timing of onset of symptoms. Genetic manipulation of the mouse is constantly being refined and a future possibility is to be able to switch genes on or off at different stages of the animal's life – a critical step in the study of late onset neurodegenerative diseases. As a result of the experimental refinements made possible by what is known as the 'genotype driven approach', gene therapies for ALS and other motor neuron degeneration diseases (all of which are incurable) have begun to be developed.

Reduction

43. A requirement of the Animals (Scientific Procedures) Act 1986 is that the smallest number of animals should be used, consistent with achieving the objectives of the experiment. The number needed will depend on the variability of the animals, the minimum size of any statistically significant difference between treatment groups and the chances of obtaining misleadingly negative conclusions.

44. Using animals of similar age, weight, genetic composition and so forth can reduce variability of the animals. Best use of the animals can be obtained by appropriate experimental design and by correct statistical analysis of the data. Failure to use good design will result in more animals being used than is necessary. Poor statistical analysis will result in unnecessary waste because the conclusions are unreliable or the data are not used as productively as might otherwise have been the case. This means that researchers must be properly trained in statistics and should receive advice from a statistician, who is experienced in dealing with those who work with animals.

45. Sound experimental design and forethought about statistical analysis are the first steps to reducing the number of animals used in any procedure. Achieving a reduction in the number of animals used is more difficult, however, when a small number of experimental subjects would have been used anyway, for example in research using non-human primates. The reduction process, like refinement, is led by generic questions: How can more data per animal be obtained? Can the experiment be repeated with the same research subjects experiencing a variety of experimental variables?

Case study on reduction

46. The introduction of new techniques can help reduce the numbers of animals used. In experiments to investigate brain function, for example, a region of brain tissue may be permanently removed by surgical lesion in an attempt to understand its function. It may be possible, however, to use reversible chemical lesions, or temporarily interfere with brain function through cooling by injecting chilled saline into the study area (Lomber 1999) to decrease the numbers of animals used. Brain images of animals also provide information about the area under study, and may help to reduce the need for more invasive investigative methods. It may also be possible to use advanced surgical techniques that obviate the need to kill the animal, as seen in the use of partial lesions to produce visual deficits in only one quadrant of the visual field. Scientists were thus able to use the animals as their own controls by testing their perceptual functions in both the lesioned and non-lesioned part of the visual field representation. These experiments were also able to maximise comparability with human data by training the animals in tests identical to those used with human subjects. Thus the amount, quality and comparability with human data were all enhanced at the same time as the total number of animals used was reduced.

47. Genetically modified animals may also offer new opportunities for refinement and reduction. A question often asked of animal research is how one knows whether the species being used is relevant. GM animals provide a means of ensuring that the characteristics of the system under investigation are as closely matched as possible to the human system that is the target of the research. The research and potential medical benefits of GM animals are already being pursued in research on heart disease, cancer and muscular dystrophy. From a refinement point of view, having available the most appropriate animal model increases the power of an experiment and also allows the most sensitive techniques and measures to be used. These experimental refinements can provide answers to the three questions posed above: the best choice of animal, which may or may not be GM, is a first step to minimising unwanted aspects of experimental procedures.

Replacement

48. The use of whole animals is a key element of much scientific and medical research as it enables normal physiological processes to be studied within the environment of the living body, and helps identify interactions that influence disease processes.

49. When a specific mechanism can be identified, the use of cell cultures may be possible. Similarly, molecular sensors may be used to test the biological activity of particular substances. When enough is known about a complex system found in intact animals, computer simulations could prove helpful in exploring the dynamics of that system; in effect, experiments can be conducted on the computer model. However, such studies generally suggest and require further work on whole animals and do not completely replace experiments on animals. Alternatives to whole animals are clearly versatile, but are as yet incapable of capturing the complexity of the living mammalian body. Research that would be typically carried out on animals has been conducted on humans, but when the outcome is unknown, as is usually the case in research, or the techniques are invasive, the ethical justification for such work is highly questionable – even when the human subjects are volunteers.

Case study on replacement

50. The lesion method, studying the effects on behaviour of compromising or removing tissue, is a widely used experimental system in behavioural sciences. One can, however, temporarily interfere with brain functions by applying brief magnetic pulses to transiently interfere with normal function. This method therefore replaces the need for permanent, surgical interference of brain function in some experiments. In practice, this technique, called transcranial magnetic stimulation or TMS, has now been used to study the timing of information transfer between human cortical areas, changes in brain function due to learning and a wide range of perceptual, movement and language functions.

51. This ability to use human subjects in lesion experiments now obviates the need for some lesion experiments in non-human primates. This is limited, however, to cases where the brain region of interest is accessible to surface stimulation. Many areas of interest lie too deep to be penetrated by the magnetic fields, in which case surgical lesions in animals are still necessary. A question about TMS is how one knows the right area has been stimulated with the same degree of certainty as total removal of a brain region by surgery. However, high levels of accuracy of the TMS technique can be established by co-registration of the magnetic stimulation site with a human subject's individual MRI scan. TMS also has very high temporal resolution and cortical functions can be disrupted within time windows as small as 5 milliseconds, thus allowing a temporal dimension to lesion analysis that is difficult to achieve in monkeys with other methods.

52. The principles behind these examples of the three Rs can be extended or modified to other types of animal research. Reduction would seem to be the factor on which most progress can be made by changes in experimental design and procedures. Refinement is an ongoing process dependent on new techniques and improvements in husbandry. Researchers routinely implement refinement and reduction in their daily routines – it is both good science and cost effective to improve experimental design and increase statistical power. The third R, replacement, is more difficult to achieve because of the unique insights provided by use of whole animals and because some techniques cannot of course be used with human subjects. Notwithstanding the difficulties, every effort to pursue the three Rs should be made.

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http://royalsociety.org/uploadedFiles/Royal_Society_Content/policy/publications/2004/9726.pdf

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