Polycystic kidney disease (PKD) is an inherited disease that causes water loss from the body and affects cell division in the kidneys. Because water movement across cell membranes is related to ion movement, scientists investigated the role of the \( \text{Na}^+/\text{K}^+ \) ATPase (also known as the sodium/potassium pump) in this disease. Ouabain, a steroid hormone, binds to the \( \text{Na}^+/\text{K}^+ \) ATPase in plasma membranes. Individuals with PKD have a genetic mutation that results in an increased binding of ouabain to the \( \text{Na}^+/\text{K}^+ \) ATPase. The scientists treated normal human kidney (NHK) cells and PKD cells with increasing concentrations of ouabain and measured the number of cells (Figure 1) and the activity of the \( \text{Na}^+/\text{K}^+ \) ATPase (Figure 2) after a period of time. The scientists hypothesized that a signal transduction pathway that includes the protein kinases MEK and ERK (Figure 3) may play a role in PKD symptoms.

![Figure 1. Cell number compared with the number of cells at 0 pM ouabain. Normal human kidney (NHK) cells and polycystic kidney disease (PKD) cells were treated with increasing concentrations of ouabain. Error bars represent ±2SE.](image1)

![Figure 2. Percent \( \text{Na}^+/\text{K}^+ \) ATPase activity of NHK and PKD cells treated with increasing concentrations of ouabain. Error bars represent ±2SE.](image2)

![Figure 3. Signal transduction pathway hypothesized to play a role in the increased number of PKD cells](image3)
(a) **Describe** the characteristics of the plasma membrane that prevent simple diffusion of $\text{Na}^+$ and $\text{K}^+$ across the membrane.

Accept one of the following:

- The interior of the plasma membrane is hydrophobic/nonpolar.
- The phospholipid tails are hydrophobic/nonpolar.
- The exterior of the plasma membrane is hydrophilic/polar.
- The phospholipid heads are hydrophilic/polar.

**Explain** why ATP is required for the activity of the $\text{Na}^+/\text{K}^+$ ATPase.

- The $\text{Na}^+/\text{K}^+$ ATPase pumps ions against their concentration gradients. This requires an input of (metabolic) energy.

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(b) **Identify** a dependent variable in the experiment represented in Figure 1.

- The number of cells

**Justify** the use of normal human kidney NHK cells as a control in the experiments.

Accept one of the following:

- It allows the scientists to determine the effect of PKD on the cells’ responses to (various concentrations of) ouabain.
- It allows the scientists to compare the responses of PKD cells and normal cells (to ouabain).

**Justify** the use of a range of ouabain concentrations in the experiment represented in Figure 1.

Accept one of the following:

- The scientists need to determine whether different concentrations have different effects on the cell numbers.
- The scientists did not know at which concentration of ouabain there would be an effect.

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(c) Based on the data shown in Figure 2, **describe** the relationship between the concentration of ouabain and the $\text{Na}^+/\text{K}^+$ ATPase activity both in normal human kidney (NHK) cells AND in PKD cells.

Accept one of the following:

- Increasing concentrations of ouabain result in decreasing ATPase activity (in both types of cells).
- There is an inverse relationship/negative correlation between the concentration of ouabain and the ATPase activity (in both types of cells).

The scientists determined that $\text{Na}^+/\text{K}^+$ ATPase activity in PKD cells treated with 1 pM ouabain is 150 units of ATP hydrolyzed/sec. **Calculate** the expected $\text{Na}^+/\text{K}^+$ ATPase activity (units/sec) in PKD cells treated with $10^6$ pM ouabain.

- 45 (Accept between 40 and 50)

| Total for part (c) | 2 points |

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In a third experiment, the scientists added an inhibitor of phosphorylated MEK (pMEK) to the PKD cells exposed to $10^4$ pM ouabain. Based on Figure 3, predict the change in the relative ratio of ERK to pERK in ouabain-treated PKD cells with the inhibitor compared with ouabain-treated PKD cells without the inhibitor.

Accept one of the following:

- Option 1: The ratio of ERK to pERK will increase in the cells with the inhibitor.
- Option 2: The ratio of ERK to pERK will stay the same in the cells with the inhibitor.

Provide reasoning to justify your prediction.

The justification must indicate that the pMEK inhibitor blocks further phosphorylation of ERK AND one of the following:

Option 1:

- The amount of pERK will not increase as it does in cells without the inhibitor.
- The amount of ERK will not decrease as it does in cells without the inhibitor.
- The cell continues to synthesize ERK.
- Phosphorylated ERK is being dephosphorylated to ERK.

Option 2:

- No additional ERK is synthesized/pERK is not being dephosphorylated.

Using the data in Figure 1 AND the signal transduction pathway represented in Figure 3, explain why the concentration of cyclin proteins may increase in PKD cells treated with $10^4$ pM ouabain.

- The cell number increases to a maximum at $10^4$ pM ouabain. The signaling pathway stimulates transcription of genes involved in cell division. The target genes likely include those for cyclins because cyclins regulate the cell cycle.

Total for part (d) 3 points

Total for question 1 10 points
The plasma membrane is composed of phospholipids which have a hydrophilic head and hydrophobic fatty acid tails. This plasma membrane is semi-permeable, meaning that only nonpolar small substances can undergo simple diffusion through this membrane. Since Na⁺ and K⁺ are ions, they have an electric charge. They must enter the cell through transport proteins since they can't diffuse through the membrane. Because cells create a gradient of K⁺ ions within the cell, ATP is required for the transport protein Na⁺/K⁺ ATPase. Since the cell is bringing in more K⁺ ions against their concentration gradient and pumping out Na⁺ ions, a gradient of ATP energy is necessary for the protein to work.

Dependent variable is the number of resulting NIH and PKD cells following different levels of ouabain in NIH cells serve as a model to compare the cellular changes caused by ouabain in PKD cells. Since the values collected would be insignificant without a relevant comparison, using a range of ouabain concentrations allowed the researchers to observe the changes that occur in cells at multiple conditions. Since a very small amount of ouabain may have a very different effect on kidney cells compared to a very high amount.

In both normal kidney cells and PKD cells, as concentration of ouabain increases past 10⁻⁴ M, a drastic decrease in Na⁺/K⁺ ATPase occurs. So there is a negative relationship between ouabain and Na⁺/K⁺ ATPase activity. In PKD cells treated at with 10⁻⁶
pM oocahan, Na+/K+ ATPase activity is 45 units of ATP hydrolyzed per second. The relative ratio of ERK to pERK will decrease in cells treated with the inhibitor compared to cells not treated with the inhibitor. Since pMEK signals the transfer of ERK to pERK, if this pERK is inhibited, it won’t allow for the phosphorylation of ERK to pERK and the amount of ERK will increase relative to pERK, causing an increase in ratio.

Ouabain is a signaling molecule that causes the transcription of target genes in 2-hybrid cells. Since these cells have increased binding of ouabain, and cells in a concentration of 10⁻⁴ ouabain have significantly higher levels of ouabain, it can be inferred that ouabain increased transcription of cyclin genes which promote cell growth and division, as shown in Figure 1.
a) Na\textsuperscript{+} and K\textsuperscript{+} are charged ions. Since they have the positive charge, they will be unable to diffuse through the plasma membrane. ATP is required for the activity of the Na\textsuperscript{+}/K\textsuperscript{+} ATPase because this represents a form of active transport. Active transport requires energy, and the ATP molecule provides this.

b) The dependent variable in Figure 1 is the number of cells. NITK cells are important as controls because they allow the scientists to see the differences PK1 cells have when compared to the NITK cells. In other words, the NITK cells provide a benchmark for comparison. The range of ouabain concentration serves as an independent variable in this experiment. By changing the ouabain concentration, the scientists are able to see the effect the concentration has on the number of cells.

c) As the concentration of ouabain increases, the Na\textsuperscript{+}/K\textsuperscript{+} ATPase activity gradually decreases. Expected Na\textsuperscript{+}/K\textsuperscript{+} ATPase activity in PK1 cells treated with 10^{-5} M ouabain is: \( \frac{3}{10} \times 15 = 4.5 \) units/second.
Continue your response to **QUESTION 1** on this page. Do not skip lines.

d) The relative ratio of ERK to p-ERK in untreated PKD cells with the inhibitor will increase because the production of MEK is needed for the production of p-ERK. The concentration of cyclin proteins may increase in PKD cells because the signal transduction pathway will create more DNA which can be transcribed and then translated into cyclin proteins.
Begin your response to QUESTION 1 on this page. Do not skip lines.

(A) When things go across the plasma membrane they do not diffuse together correctly which is why simple diffusion is prevented. The reason ATP is needed for the activity is because ATP is what allows the binding of Na⁺ and K⁺.

(B) The number of cells is dependent on the amount of Ouabain. The NHK cells should be the control because even with the increasing concentrations they remained near the same levels and did not vary too much like the PKD cells did. The reason the Ouabain range was so large was because they wanted to see the reaction that would happen over greater increases.

(C) The less Ouabain concentration the greater the Na⁺/K⁺ activity will be. The percent activity would be 36%.

(D) If pMek was added it would throw off the ratio because there would be 3 MEK to 2 ERK. If there was a cyclin increase then PKD cells would increase because cyclin is a part of PKD cells.
Question 1

Note: Student samples are quoted verbatim and may contain spelling and grammatical errors.

Overview

This question was based on polycystic kidney disease (PKD), which results in water loss from the body and affects cell division in kidney cells. The question described two experiments in which normal human kidney (NHK) cells and PKD cells were treated with increasing concentrations of the steroid hormone, ouabain. Experimental results were presented in two graphs: Figure 1 showed the number of NHK and PKD cells in various ouabain concentrations, and Figure 2 showed the percent $\text{Na}^+ / \text{K}^+$ ATPase activity in the various ouabain treatments. A third figure illustrated a hypothesized signal transduction pathway initiated by ouabain binding to $\text{Na}^+ / \text{K}^+$ ATPase.

In part (a) students were asked to describe characteristics of the plasma membrane that prevent simple diffusion of $\text{Na}^+$ and $\text{K}^+$ across the membrane and to explain why ATP is required for the activity of the $\text{Na}^+ / \text{K}^+$ ATPase. Responses were expected to demonstrate an understanding of the structure of plasma membranes (Topic 2.4 in the AP Biology Course and Exam Description), membrane permeability (Topic 2.5), active transport (Essential Knowledge ENE-2.E.3 in Topic 2.6), and the function of the $\text{Na}^+ / \text{K}^+$ ATPase (ENE-2.G.4 in Topic 2.7).

In part (b) students were asked to identify an independent variable in the experiment represented in Figure 1, to justify the use of NHK cells as a control, and to justify the range of ouabain concentrations used. Responses were expected to demonstrate proficiency in identifying experimental procedures (Science Practice 3.C).

In part (c) students were asked to describe the relationship between concentration of ouabain and $\text{Na}^+ / \text{K}^+$ ATPase activity in both NHK and PKD cells. Responses were expected to demonstrate proficiency in describing data from a graph (Science Practice 4.B). Students were also asked to perform a mathematical calculation based on the data presented (Science Practice 5.A).

Part (d) described a third experiment in which scientists added an inhibitor of one of the components of the signal transduction pathway. Students were asked to predict the effect of the inhibitor and justify their prediction. Responses were expected to demonstrate an understanding of signal transduction pathways (Topics 4.2, 4.3, and 4.4 of the CED) and proficiency in scientific argumentation (Science Practices 6.E and 6.C). Finally, students were asked to explain why the concentration of cyclin proteins may increase in PKD cells treated with a specific concentration of ouabain. Responses were expected to demonstrate an understanding that the signaling pathway stimulates gene expression (IST-3.D.2 in Topic 4.2) and that cyclins regulate the cell cycle (IST-1.D.1 in Topic 4.7).

Sample: 1A
Score: 10

The response earned 1 point in part (a) for describing the hydrophilic heads and … hydrophobic tails as characteristics that prevent simple diffusion of the ions. The response earned 1 point in part (a) for explaining the movement of ions against their concentration gradient and the use of energy from ATP. The response earned 1 point in part (b) for identifying the number of cells as the dependent variable. The response earned 1 point in part (b) for justifying the use of NHK cells as a basis of comparison to PKD cell. The response earned 1 point in part (b) for justifying the use of a range of ouabain to the different effects on cells in different conditions (concentrations of ouabain). The response earned 1 point in part (c) for describing the inverse relationship between ouabain concentration and ATPase activity. The response earned 1 point in part (c) for the correct calculation of $\text{Na}^+ / \text{K}^+$ ATPase activity as 45. The response earned 1 point in part (d) for predicting the increase in the ratio of ERK to pERK in cells treated with the inhibitor correctly. The response earned 1 point in part (d), justification, for addressing the role of pMEK in phosphorylation of ERK correctly and the increase in ERK that results. The
Question 1 (continued)

response earned 1 point in part (d) for explaining ouabain causes the signaling pathway, which stimulates transcription of target genes (cyclin genes) involved in cell growth and division as shown in Figure 1 (cell number).

Sample: 1B
Score: 6

The response did not earn a point in part (a) because it describes characteristics of the ions not the membrane. The response did not earn a point in part (a) because it does not explain the movement of ions against their concentration gradient. The response earned 1 point in part (b) for correctly identifying the dependent variable. The response earned 1 point in part (b) for justifying the NHK cells as a control because “they allow scientists to see the differences” when comparing the cell types. The response earned 1 point in part (b) for justifying the use of a range of ouabain to see the “effect the concentration has on the number of cells.” The response earned 1 point in part (c) for correctly describing the relationship between ouabain concentration and \( \text{Na}^+ / \text{K}^+ \) ATPase activity. The response earned 1 point in part (c) because it correctly calculates the value as 45. The response earned 1 point in part (d) by providing a proper prediction. The response did not earn a point in part (d) because it does not justify the concentrations of ERK or pERK changing. The response did not earn a point in part (d) because it does not use the data and does not explain the signal transduction pathway in Figure 3.

Sample: 1C
Score: 3

The response did not earn a point in part (a) because it does not describe characteristics of the membrane. The response did not earn a point in part (a) because it does not explain that \( \text{Na}^+ \) and \( \text{K}^+ \) are moved against their concentration gradients, requiring energy. The response earned 1 point in part (b) for identifying the number of cells. The response did not earn a point in part (b) because it does not justify the use of NHK cells as a control. The response earned 1 point in part (b) for justifying that the scientists “wanted to see the reactions that would happen [effects] over greater increases [in concentration].” The response earned 1 point in part (c) for describing that “The less ouabain concentration the greater the \( \text{Na}^+ / \text{K}^+ \) activity will be.” The response did not earn a point in part (c) for the calculation. The response did not earn any points in part (d).