Part 1: Scientific Report

Investigation on Alzheimer 's Disease

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Synopsis

In this scientific investigation, I pursued the following hypothesis and associated research questions:

- ***Hypothesis 1:*** *that a genetic or non-genetic imbalance of sugar in brain fluid could contribute to the osmotic destruction of brain tissue and possible prion accumulation observed in Alzheimer's disease.*
- ***Research Question 1:*** *What is the impact of alcohol, salt and sugar solutions on the structure of lamb's brains?*
- ***Research Question 2:*** *Could osmosis be responsible for the destruction of living cells?*
- ***Research Question 3:*** *Is it feasible that prions get entrapped in a damaged Alzheimer's brain?*

Acknowledgement

I wouldn't have done this investigation if the STANSW Young Scientist competition didn't exist, and without the encouragement of my classroom teacher. I am very grateful for this opportunity, and thrilled with what I was unable to uncover.

My grandma and mum helped me with the experimental setup, the hypothesis and the research questions. Mum also helped me with the structure of this write-up, importing the photos, and organising the data. None of us were at all sure where we were heading at the beginning, but by the time we'd finished, we'd all learnt a great deal.

Dedication

Many people and families around the world would like to break the curse of Alzheimer's[i], crack the spell, and find a cure, and I dedicate this work to them.

Background

My hardworking and highly successful maternal grandpa died in March this year at age 75 after more than eight years of a severe, steady and devastating decline to Alzheimer's[ii] disease. At first he lost his short term memory, then his ability to comprehend what people were saying to him, and then his ability to read. Next, his long term memory failed him, his cognition failed him, and he couldn't draw sensible conclusions. He started getting lost, and doing strange things around the house, and in public. He started hallucinating, and talking complete nonsense. After we got him into high-care, they had to chemically sedate him, and then his bodily systems started failing. Eventually he died with a heart attack in the nursing home. It was a horrible, undignified and tragic way to go. It was exceptionally hard on grandma.

Nobody knows why Alzheimer's[iii] happens but it's more severe than regular age-related dementure, and absolutely crippling. As a family, we felt hopeless to do anything about it. My grandma had heard that Alzheimer's could be some kind of diabetes-related disease in which the body fails to properly process sugar[iv], and that in some cases, self-replicating prions could be involved.

Presently there is absolutely no cure and little relief for Alzheimer's sufferers and their families. Some families have the Alzheimer's gene. If you personally have got that gene[v], you've got almost a 100% chance of getting the disease. Some people are born with Type 1 Diabetes, so it seems feasible that a genetic type of brain diabetes that leads to Alzheimer's could be possible.

Aim and Hypothesis

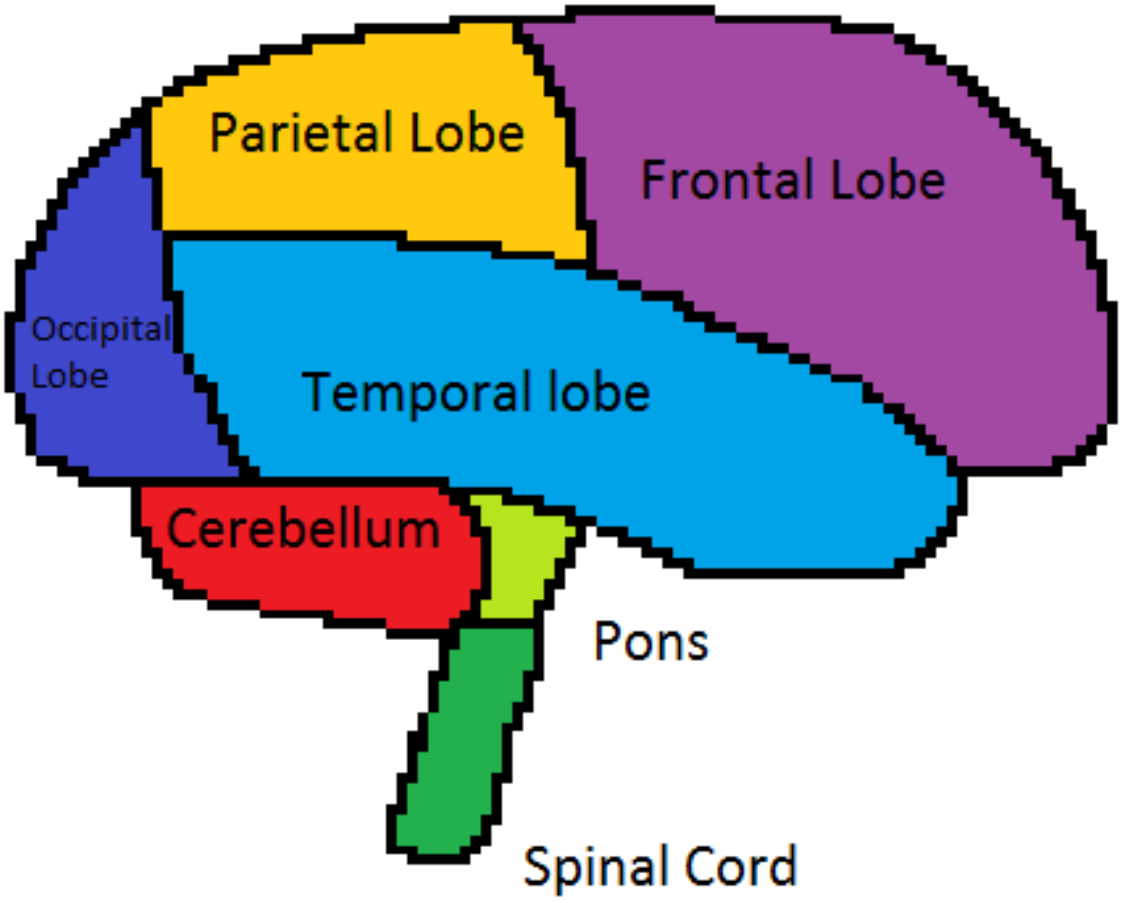
The aim of this scientific investigation was to explore the link between brain biochemistry and Alzheimer's, and make a suggestion for a cure. My scientific hypothesis for this investigation was:

Hypothesis 1: *that a genetic or non-genetic imbalance of sugar in brain fluid could contribute to the osmotic destruction of brain tissue and possible prion accumulation observed in Alzheimer's disease.*

Preparation

I searched the Internet with my mum, and we found that I could purchase a slide of a mouse brain from a science supplies store in Melbourne to get an idea about how to create our own slides to investigate Alzheimer's. We rang up the Australian Museum to see if we could use their microscopes during the investigation, and the manager of the Search and Discover centre agreed. My mum helped me order the mouse brain slide, and we had it posted to Sydney, along with some disposable scalpels, and some blank slides.

Here is a picture of a human brain that I drew on Microsoft Paint, after researching brains on the Internet[vi]:



Knowing that mice, lambs and humans are all mammals, we got to thinking that we could use lambs brains from the local butcher to pursue the investigation. I went to our butcher and bought a tray of frozen lambs' brains, which I took home, and stored in the freezer. There were 5 brains in the tray. I wasn't too worried about working with the brains because people actually eat them, however my mum was freaked out. We researched the Animals in schools policy about dissection and disposal here: http://nswschoolanimals.com/index/dissection-of-animals/ and here: http://nswschoolanimals.com/index/disposal-of-animals/ to make sure that we would be compliant. Since the animal parts used are foodstuffs from a local butcher, and there were no special constraints made on disposal, we were compliant.

Special preservatives for brain tissues, slide die, and slide glue were expensive from the science supplier, and in some cases cancer causing and restricted, so I needed to improvise with my supplies.

Mum found some Vodka left over from a party that was 37.5% alcohol and suggested we use it for preservative. The high alcohol content meant that it can kill a lot of different types of bugs. We got the red food colouring from a kitchen cupboard in case we needed to dye the slides. We also bought some superglue from the hardware to glue our slides together. We were excited when the scalpels, blank slides, and the prepared mouse brain slide arrived, although they took a while to get here.

We reviewed the Young Scientists Ethics form, but didn't request anyone to sign it since the background to the research was personal, and no interviews with human subjects were required.

Research Questions

As the investigation progressed, we were able to explore a number of research questions.

Research Question 1

We got to thinking that alcohol, salt and sugar are all bad for the body, and especially the brain, so, we decided to investigate the impact of different solutions, respectively comprising alcohol, salt and sugar on mammalian brain tissues.

Mum knew we could use the Vodka for the alcohol, plus boil up some sugar and salt solutions. In pursuit of my research aim and hypothesis presented earlier, this led to my first research question:

Research Question 1: *What is the impact of alcohol, salt and sugar solutions on the structure of lamb's brains?*

Research Question 2

Once we saw the impact of the three solutions on the brain tissue, and I talked to my grandma who used to be a biologist and a zoologist, I wanted to understand chemical osmosis better. Grandma told me that sugar is a really big molecule that doesn't pass through mammalian cell membranes, so to restore osmotic equilibrium, it sucks water out of neighbouring cells. In contrast, salt splits into tiny sodium Na+ and Chlorine Cl- ions in solution that can pass through the cell walls of brain tissue. Similarly, components of alcohol are able to permeate living tissue and cells. This understanding led to the following second research question:

Research Question 2: *Could osmosis be responsible for the destruction of living cells?*

Research Question 3

Once mum and I saw the devastating effects that the osmotic pressure resulting from a variety of chemicals can have on living tissue and cells, including jellification, death, and shrinkage, we were struck by a third research question:

Research Question 3: *Is it feasible that prions get entrapped in a damaged Alzheimer's brain?*

Risk Assessment

We got to thinking about the risks involved, and we bought some safety equipment including gloves and masks. Below is a table of our anticipated risks and how we mitigated them:

Procedure	Risk	Solution
Working with the brain tissues	Biological risk of decay and hence transmissible disease	Gloves, Masks, Aprons (lab-coats), disposable trays, spoons, containers, and plenty of rubbish bags
Cutting the brain tissues	Cuts, transfer of disease	We bought and used our made-for-purpose long blade disposable scalpels
Preparing the slides	Exposure to biological tissues and potential for superglue on the skin	We used gloves, masks, and a number of different small disposable tubes of glue
Boiling up different solutions	Burns, Fire	Mum did the boiling in the kitchen, everyone else doing their own things

My mum gave me permission to work in the laundry, storing the brains in the garage as we progressed. We used aprons for lab-coats and wore gloves and masks at all times, being careful not to inadvertently transfer tissues or bugs, and frequently disposing of the waste into plastic bags.

This was our laundry setup:

This was our garage setup:

Here are the gloves, and disposable scalpels we used:

General Method

On the first day of my investigation, I thawed the 5 lamb's brains and had a look and feel of them to figure out what we could do with them. I have included the photos that I took in my electronic logbook, which I have included with this scientific report.

With 5 brains, we would have 10 brain hemispheres, each brain with a left and a right hemisphere. I looked at the different parts of them, and figured out what was what by searching on the Internet.

I wanted to make sure that we could try three different brain samples in each of our three different solutions, so I could have some confidence in my results, so mum and I decided to split the 5 brains with their combined 10 brain hemispheres into 3 hemispheres in each of 3 separate alcohol, sugar and salt solutions, leaving 1 hemisphere for dissection and slide making.

That meant that we would run separate scientific investigations on the brain, over a number of days, with a number of trials in parallel, one with brain hemispheres in solution, and one with slices of brain hemisphere on slides.

We went to Woolworths and bought a number of same-sized containers to soak the brain hemispheres in. We picked three different colours: pink, blue and green for the three different solutions, and numbered the containers from 1 to 9, allowing 1 left over purple container to hold the slides from the 10th dissected brain hemisphere.

To measure any potential brain shrinkage, as is observed in the brains of late-stage Alzheimer's sufferers, we put some 2mm grid paper inside plastic sleeves to lay the brains out on, before and after our experimental trials, so we could easily measure the height, width, and potentially the cross-sectional area of the hemispheres.

Method in Pursuit of Research Question 1

As before, our first research question was:

Research Question 1: What is the impact of alcohol, salt and sugar solutions on the structure of lamb's brains?

In pursuit of an answer to this question, we defrosted the Vodka that dad had stored in the freezer, and cooked up separate sugar and salt solutions.

For the sugar solution (rear left), mum boiled one cup of table sugar into one cup of water in a pot on the stove until the sugar fully dissolved, leaving a >50% saturated sugar solution. It took about 10 minutes.

With the salt solution, the table salt didn't want to dissolve into its water as well as the sugar had (back left). We started off with 1 cup of salt to 1 cup of water, but had to double the water to get the salt to dissolve. Heat didn't seem to help the salt dissolve in the same way as had helped for the

sugar, and after about 10 minutes we ended up with a 25% saturated salt solution (front right), but you could still see some of the salt crystals, some of which we left behind in the original pot (front left).

As shown in the following example, we photographed each of the brain hemispheres on the grid paper (for measurement) before putting them in solution:

We figured out a fair test with the brains by making a matrix of brains, hemispheres and solutions, and we mapped out our allocation in a table in my handwritten logbook (please see attached electronic logbook which records these mappings with photos of the hemispheres).

After taking the photos, we spooned the first nine hemispheres (matching every column and row of our table) into the three different coloured coordinated solutions as follows, and left them over several nights to soak, photographing and measuring them as time progressed:

Container Column 1: Green	Container Column 2: Blue	Container Column 3: Pink
Vodka 37.5% alcohol	Sugar Solution >50% sugar saturated	Salt Solution 25% salt saturated

The results are recorded in my electronic logbook, which I hope you will now refer to.

Method in Pursuit of Research Question 2

Once we saw the impact of the three solutions on the brain tissue, mum and I got to asking grandma about osmosis, and our second research question became:

Research Question 2: *Could osmosis be responsible for the destruction of living cells?*

We asked Nanna how to setup a test for this question, and she suggested that we get a soft plant like lettuce, or a soft leaf, and dunk it in each of our three different solutions to see what would happen.

Osmosis[vii] happens when chemicals in solution on each side of a membrane want to equalise, lowering their potential energy to reach chemical equilibrium, but some of the molecules are too big to pass through. In that case, instead of the big molecules crossing the membrane, which they can't, smaller molecules like water actually pass the other way.

Day 3 | Date: 20/8/2015, Time: 8:00pm

We took three fern fronds with multiple frond fingers from a tree fern in the backyard, labelled three plastic cups, and filled them with the leftover exact same solutions that we had prepared for the brains: vodka, sugar and salt. We left the fern fronds in the solutions overnight, and checked back the following day, to see the results. We then repeated the test with three additional fern fronds in each solution.

Again, the results are recorded in my electronic logbook, which you can refer to here.

Method in Pursuit of Research Question 3

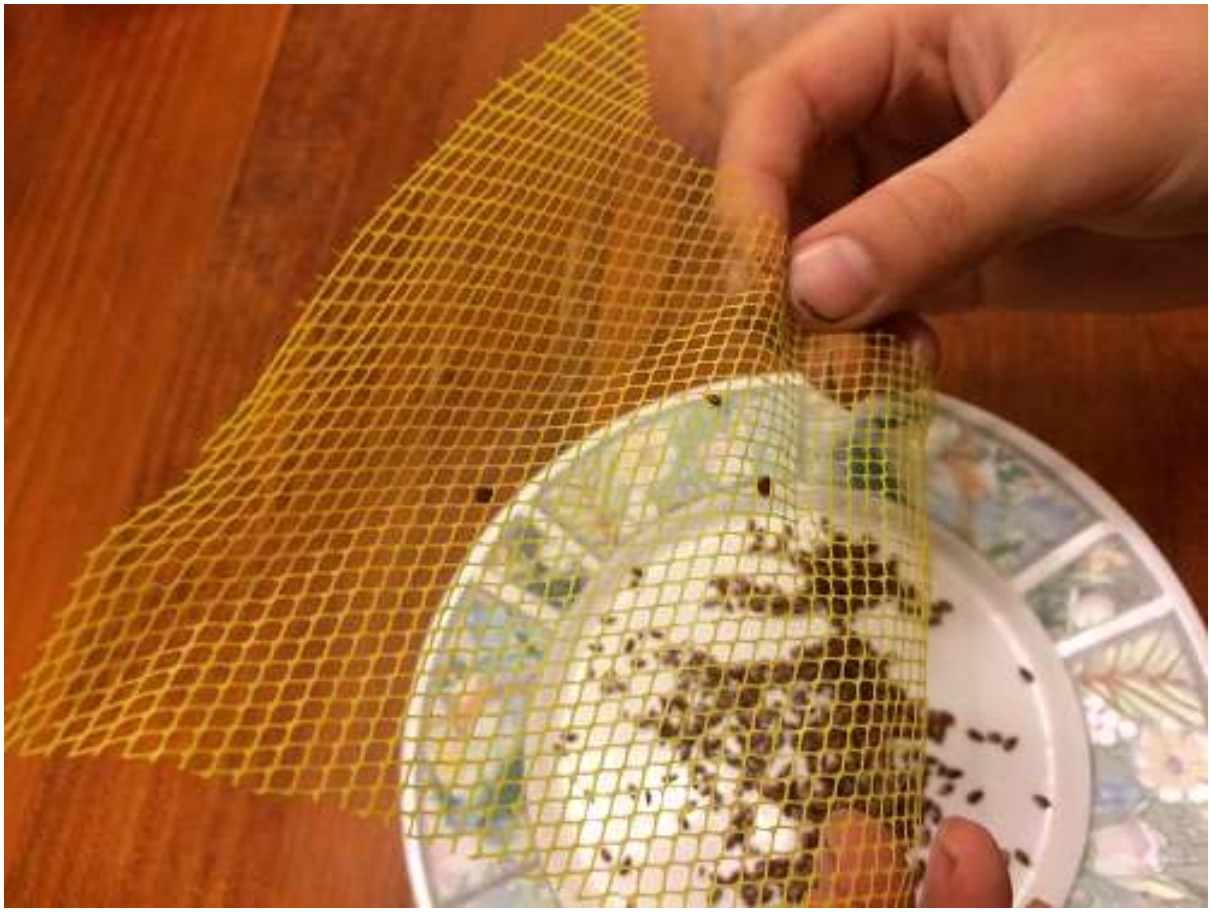
Mum found out from the Internet that prions can be sizeable[viii], and from our investigations, we began to suspect that body and brain chemistry might play a really big role in Alzheimer's, and in trapping unwanted particles like prions. Our third research question became:

Research Question 3: *Is it feasible that prions get entrapped in a damaged Alzheimer's brain?*

Medical researchers in the past have conjectured that prions could cause Alzheimer's disease because of their discovered presence in the brains of some diseased Alzheimer's sufferers. However, there is an old scientific adage that *correlation is not causation*. Just because prions in the brain tissue appear to be *correlated* with Alzheimer's, it doesn't mean that they *cause* Alzheimer's.

Mum found out that prions were of a certain size, so we had the idea to find and cut up a net that holds lemons, like the sort of net that holds oranges, to demonstrate how particles like bacteria, prions or proteins could have a greater likelihood of entrapment if the brain tissue became smaller, stickier or damaged, like a fine grained net, and/or when the particles themselves are substantial.

In the photo below, I used linseeds in a lemon net to illustrate:



With the spare 10th brain hemisphere, mum and I decided to make three columns of 5 rows of slides with brains and our spit on them, hopefully including some bacteria or even prions, to be covered up over several days. Slides 1, 2, and 3 would be covered up immediately, and the later slides on later days so they could sit be exposed to the atmosphere for a little while to help them decompose.

One column would have no saliva and just brain tissue that had been left out a while. The second column would have brain tissue in my spit. The third column would have brain tissue in mum's spit. We would cover up the different rows on different days.

We didn't want to generate or spread any infection, so we were extra cautious with this part in respect of gloves, masks, aprons, and securing the specimens in a garbage back, so as not to cause a zombie apocalypse.

Summary of Results

I kept a hand-written logbook and a photo record to capture my observations and results during the course of my scientific investigations, and I reproduced my handwritten observations and photos in my electronic logbook.

The following sections rely upon my electronic logbook, included with this scientific report, to present a summary of the results of my investigations into research questions 1, 2 and 3.

Summary of Results for Research Question 1

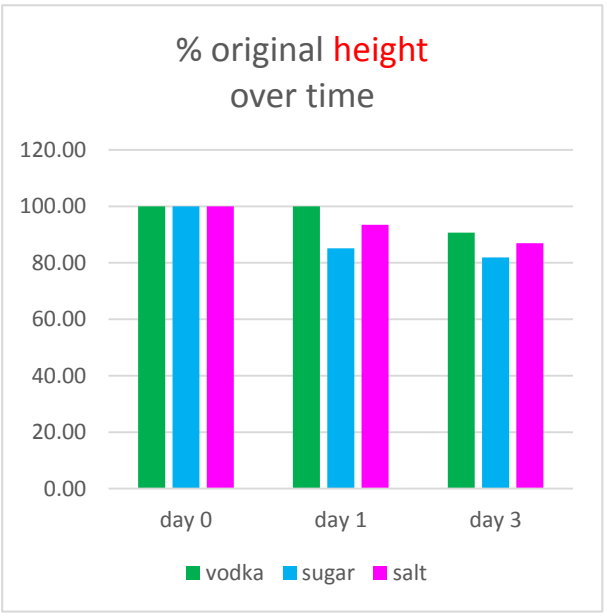
Research Question 1: *What is the impact of alcohol, salt and sugar solutions on the structure of lamb's brains?*

Using the grid paper, we measured the percentage change in the cross-sectional height and width of each brain hemisphere over time, and estimated the cross-sectional area as being proportional to the height * width. The following tables and charts show the results we got:

Height (cm)

sample	day 0	day 1	day 3
1	7.00	7.00	6.25
2	7.00	7.00	6.25
3	7.50	7.50	7.00
avg vodka	**7.17**	**7.17**	**6.50**
%	**100.00**	**100.00**	**90.70**
4	8.00	6.50	6.25
5	8.00	6.50	6.75
6	7.50	7.00	6.25
avg sugar	**7.83**	**6.67**	**6.42**
%	**100.00**	**85.11**	**81.91**
7	8.00	8.50	8.00
8	7.50	6.50	6.00
9	7.50	6.50	6.00
avg salt	**7.67**	**7.17**	**6.67**
%	**100.00**	**93.48**	**86.96**
Average %	**100.00**	**92.86**	**86.52**

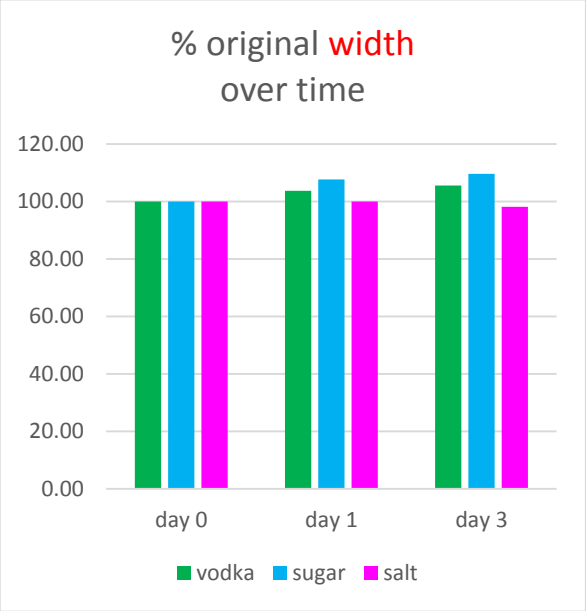
	day 0	day 1	day 3
vodka	100.00	100.00	90.70
sugar	100.00	85.11	81.91
salt	100.00	93.48	86.96



Width (cm)

sample	day 0	day 1	day 3
1	4.50	4.50	4.25
2	5.00	5.00	4.50
3	4.00	4.50	5.50
avg vodka	**4.50**	**4.67**	**4.75**
%	**100.00**	**103.70**	**105.56**
4	4.00	4.50	4.25
5	4.00	4.50	5.00
6	5.00	5.00	5.00
avg sugar	**4.33**	**4.67**	**4.75**
%	**100.00**	**107.69**	**109.62**
7	5.00	4.50	4.25
8	4.00	4.50	4.50
9	4.50	4.50	4.50
avg salt	**4.50**	**4.50**	**4.42**
%	**100.00**	**100.00**	**98.15**
Average %	**100.00**	**103.80**	**104.44**

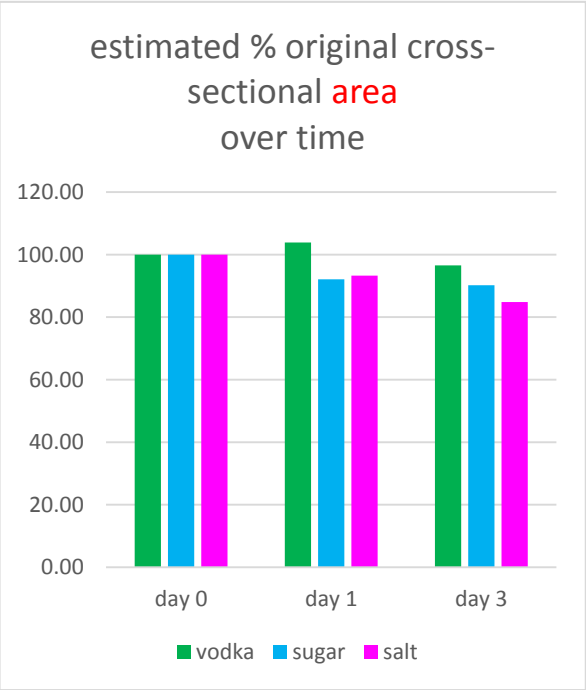
	day 0	day 1	day 3
vodka	100.00	103.70	105.56
sugar	100.00	107.69	109.62
salt	100.00	100.00	98.15



Area (cm^2)

sample	day 0	day 1	day 3
1	31.50	31.50	26.56
2	35.00	35.00	28.13
3	30.00	33.75	38.50
avg vodka	**32.17**	**33.42**	**31.06**
%	**100.00**	**103.89**	**96.57**
4	32.00	29.25	26.56
5	32.00	29.25	33.75
6	37.50	35.00	31.25
avg sugar	**33.83**	**31.17**	**30.52**
%	**100.00**	**92.12**	**90.21**
7	40.00	38.25	34.00
8	30.00	29.25	27.00
9	33.75	29.25	27.00
avg salt	**34.58**	**32.25**	**29.33**
%	**100.00**	**93.25**	**84.82**
Average %	**100.00**	**96.42**	**90.53**

	day 0	day 1	day 3
vodka	100.00	103.89	96.57
sugar	100.00	92.12	90.21



In summary, and on average:

- The vodka brains got about 10% shorter and 5% fatter, but remained about the same cross-sectional area. They were grey in colour, but still well-defined. They sank in solution.
- The sugar brains got about 20% shorter and 10% fatter, and were about 10% less in cross-sectional area by the morning of the third day. They seemed turned to jelly, shrinking and losing their structure while clearly losing blood content. It was like the sugar was pulling the goodness out of the brain. They floated in solution, and appeared greatly reduced.
- The salt brains got about 15% shorter and 5% fatter, and were about 15% less in cross-sectional area on the morning of the third day. They seemed well defined, but shrunken and curled up. They floated in solution, and appeared greatly reduced.

Summary of Results for Research Question 2

Research Question 2: *Could osmosis be responsible for the destruction of living cells?*

In summary, 16 hours, 21 and 36 hours after soaking:

- The fern fronds soaked in vodka sucked up the alcohol and almost completely died, while maintaining their structural integrity.
- The fern fronds soaked in sugar lost significant structural integrity, progressively shrinking with the leaves drooping all over the place and becoming jam- or jelly- like where contact was made with the solution, in a similar response to the brains.
- The fern fronds soaked in salt also responded in a similar way to the brains, remaining stiff but progressively curling up and shrinking.

As only the stalks of the plants were placed in the solutions, and the fern fronds visibly sucked up those solutions (until some of them fell in), it appeared that the destruction of living cells was therefore due to chemical osmosis.

Summary of Results for Research Question 3

Research Question 3: *Is it feasible that prions get entrapped in a damaged Alzheimer's brain?*

Logically, given the compression, shrinkage and destruction that we saw in the lamb's brains affected by sugar and separately salt, it seems feasible that elements in the bloodstream, such as prions that potentially circulate through a mammalian brain damaged by Alzheimer's, could become trapped. However, our results were inconclusive for Research Question 3.

We could see some artefacts in slides 11, 12 and 15, but we were unsure whether these were pathogens carried by saliva, and would need better slides, and stronger microscope magnification to pursue this line of inquiry.

Overall Analysis

In general, the scientific investigation went exceptionally well, with many interesting results. I realised the impact that sugar, salt, and vodka could potentially have on our bodies and brains.

Analysis for Research Question 1

Research Question 1: *What is the impact of alcohol, salt and sugar solutions on the structure of lamb's brains?*

All in all, it didn't look like any of the three solutions were good for lamb's brains. Sugar appeared completely devastating, causing the greatest structural impact by turning the brains to mush. The sugar soaked brains looked and felt like jam or jelly, where-as the vodka and salt soaked brains were still quite stiff and structurally intact. The sugar and salt soaked brains shrank, floated, and appeared significantly compressed as a result of their relatively low density. You wouldn't get the same concentrations of the alcohol, sugar and salt solutions that we used for this experiment in the human body, but it seems feasible that you could experience an alcohol, sugar and/or salt induced impact on your living tissues that could accumulate over time. Our experiment was an attempt to illustrate what might happen if you sped up the decay process.

Analysis for Research Question 2

Research Question 2: *Could osmosis be responsible for the destruction of living cells?*

Through the experiment with the tree fern fronds, after only a very short while it was apparent that all three solutions: vodka, sugar and salt could destroy living cells, in this case plant cells. The surprising aspect was that the tree fern fronds were destroyed in a strikingly similar manner as the brain tissue, by each respective solution. Vodka appeared to permeate and kill the living cells without affecting their structure, sugar destroyed the structure, curling and shrinking the leaves and and turned leaves to jelly on contact, and salt also made the leaves curl up and shrink.

Analysis for Research Question 3

Research Question 3: *Is it feasible that prions get entrapped in a damaged Alzheimer's brain?*

As found through research question 1, the sugar soaked brains were so shrunken and sticky, it wouldn't be surprising if biological particles like prions could get trapped inside or stuck to the sugar affected areas. The salt soaked brains also shrank, so they could possibly become prion or protein traps too. As well, the brain hemispheres were significantly compressed due to their comparatively low density and floating when suspended in the sugar and salt solutions. The floating and resultant tissue compression would seem to increase the chance of particle entrapment by the brain tissue.

A friend of my mum's is a microbiologist and she advised us that we would need at least 1000x magnification to see cells, bacteria, viruses or prions, and that the professionals preserve slides with wax, and drop coloured dye and oil and on the tissue surface to give visual definition to the examined cells. This was beyond the reach of the facilities we had for our investigation.

Our results were therefore inconclusive for Research Question 3. Logically, given the compression, shrinkage and destruction that we saw in the lamb's brains affected by sugar and separately salt, it seems feasible that larger elements that circulate through a mammalian brain damaged by Alzheimer's could become trapped, but further investigative work is needed to answer this research question.

Conclusion for my Research Hypothesis

Hypothesis 1: *that a genetic or non-genetic imbalance of sugar in brain fluid could contribute to the osmotic destruction of brain tissue and possible prion accumulation observed in Alzheimer's disease.*

From my investigation into research question 1, I have concluded that imbalances of sugar in brain fluid could impact the structure of the brain over a long period of time as is speculated for Alzheimer's disease. From my investigations into research question 2, it was clear that osmotic pressure from chemicals like vodka, sugar and salt solution can kill living cells. From my investigations into research question 3, it would seem that it is also feasible that prions could get trapped in sugar or salt damaged brain tissue, since I found evidence that the effect of concentrated sugar and salt solutions on plant and animal cells is to compress and shrink the tissue and cell matrix, and the effect of contact with concentrated sugar on brain tissue is to make it jellified and jam-like.

Response and Further Work

I have heard about dialysis machines for people with kidney failure, and insulin regulators for diabetes sufferers. What if we could invent a probe to go in the human brain, measuring the sugar and salt composition of the brain fluid, and regulating it to avoid dangerous concentrations of these chemicals?

Further work could include:

- Investigation as to the longer term impact of lower and more realistic concentrations of sugar and salt solutions on mammalian brain tissue.
- Further investigation of the soaked brains by dissection and examination under a 1000x or preferably an even stronger microscope.
- Examination under a strong microscope of bacterial, viral and prion cells in human saliva.
- Investigation of why salt didn't dissolve in boiling water as readily as sugar.
- Investigation into the density of brain tissue as well as sugar, salt and protein solutions.

References

The following references were helpful in my investigation:

[i] Alzheimer's Association, USA: https://www.alz.org/

[ii] Alzheimer's Australia: https://fightdementia.org.au/

[iii] Wikipedia: https://en.wikipedia.org/wiki/Alzheimer%27s_disease

[iv] Type 3 Diabetes Concept: http://www.greenmedinfo.com/blog/sugar-and-your-brain-alzheimer%E2%80%99s-disease-actually-type-3-diabetes

[v] Alzheimer's Genetics: https://www.nia.nih.gov/alzheimers/publication/alzheimers-disease-genetics-fact-sheet

[vi] Brain diagram at JP's teaching space: http://www.warracksc.vic.edu.au/groups/jp/revisions/1ae72/3/

[vii] Osmosis: http://hyperphysics.phy-astr.gsu.edu/hbase/kinetic/diffus.html

[viii] Size of prions compared to bacteria and viruses:
http://www.exo.net/~jyu/activities/life%20size.pdf