3rd Protocol
Biology SL

Finding the Reaction Rate of Catalase in a Pig's Liver

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Introduction:

The experiment was conducted in order to find out at which temperature catalase reacts best and how far the liver has to be heated up until the reaction is not going to take place because the enzyme denatures.

First of all, we put some pieces of liver in a mixer with 50 ml of water and mixed it until the liver pieces were as small as possible. With this solution, we prepared 25 test tubes, with 2 ml of liver solution in each of them.

We also prepared 6 pots of water, which were either heated or cooled with ice cube or boiling water, for 6 different temperatures, from 0,8°C to 55°C. Each time we put the test tube with the liver solution (chopped liver and water) into water with this certain temperature for 1 minute and then we added 5 drops of H_2O_2 with a pipette and waited for 5 seconds before measuring how high the foam had risen in the test tube. This gave us our results for the reaction rate of catalase in a pig's liver with H_2O_2. We assumed that the higher the foam rises, the more H_2O_2 reacts with the enzyme.

We took 3 measurements for each temperature.

Data Collection

Table1: Results for the reaction rate of liver for different temperatures, measured in a test tube.

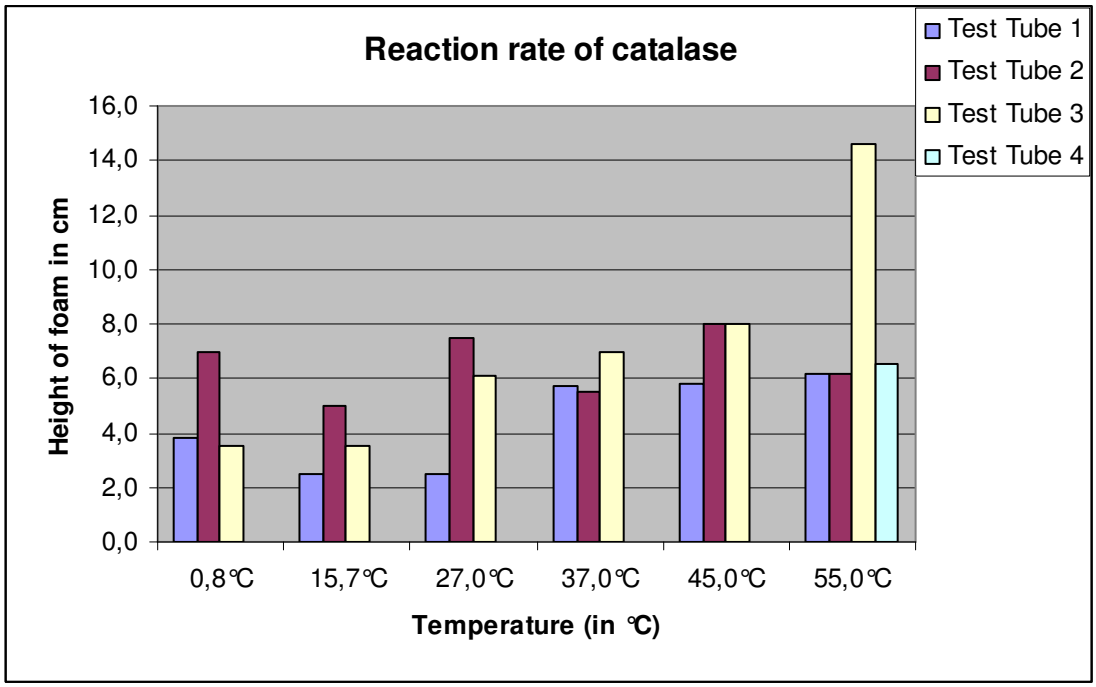
Temperature in °C	*Height of foam in cm*			
	Test Tube 1	**Test Tube 2**	**Test Tube 3**	**Test Tube 4**
0,8	3,8	7,0	3,5	
15,7	2,5	5,0	3,5	
27,0	6,3	7,5	6,1	
37,0	5,7	5,5	7,0	
45,0	5,8	8,0	8,0	
55,0	6,2	6,2	14,6	6,5

Table 1 presents the results of the various measurements. Measurements were conducted for different temperatures but with the same amount of H_2O_2 and in the same time interval. For cooling the water down to 0,8°C and 15,7°C, we used ice cubes, while for the other temperatures we cooked the water before

holding the catalase solution in the test tube into it. Then, when we had left the test tube in the water for 1 minute, we put 5 drops of H_2O_2 into the tube and after waiting for 5 seconds, we recorded how high the foam had gone.

It can be seen that for every temperature there is a test tube where the foam did not rise as high or quite a bit higher than in the other test tubes. However, at the temperature of 55,0°C, Test Tube 3 presents a result which is more than double the value of the other test tubes. It can be assumed that this test tube was a measuring error. Therefore, a fourth measurement was taken which can be seen in the column for Test Tube 4. This result is very close to the results of Test Tube 1 and 2 for this temperature and therefore we can neglect the result of Test Tube 3 at 55,0°C.

Presentation of raw data



Graph 1

According to ***Graph 1***, it seems that, on an average, the higher temperatures are better conditions for catalase to work. Nonetheless, this cannot be seen clearly, because there is always a test tube which drops out, especially for the temperatures between 0,8°C and 27,0°C.
I chose a bar graph as a representation of the data because using an x-y-graph would have produced too many confusing lines. Amongst the graphs more suitable for this purpose, the bar graph seemed best.
On the first sight, it would seem that 55,0°C are best for catalase to work, because there is a very high value of over 14°C. However, if we look at this measurement exactly, we can be fairly sure that it was an error because it is more than double as high as any of the other measurements and therefore it

can be regarded as meaningless (see explanation above). This again does not help us in drawing a conclusion as to which temperature offers best conditions. Maybe, it can be generally stated that warmer temperatures are better, because in its usual environment, a pig's body, catalase also works at body temperature. Moreover, usually at higher temperatures the molecules move faster and therefore substrates and active sites collide more easily with each other. Due to our experiment it however seems as if catalase would prefer even higher temperatures than body temperature, which seems rather illogical because at certain high temperatures, enzymes also start to denaturate.

Data processing and Presentation 1

In order to find out at which temperature catalase works best, we can calculate the averages. This way any measuring errors can be made less significant. I first calculate the average of the values I got for 0,8°C.

$$\text{average} = \frac{3{,}8 + 7{,}0 + 3{,}5}{3}$$

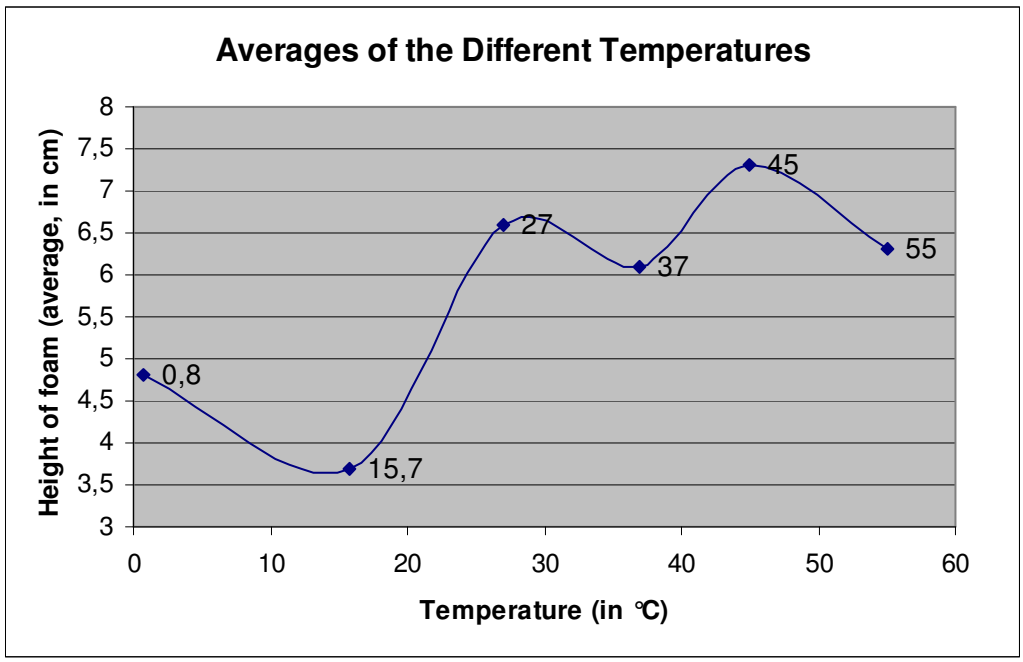
$$\underline{\text{average} = 4{,}8}$$

Using the same method we can calculate the average values for all temperatures. The only exception is that for 55°C, we do not include the value of 14,6°C for the average because, compared to the other values, this one is more than double as high and therefore doubtlessly a measuring error.

***Table 2**:* averages of the different methods calculated using the above formula.

Temperature (°C)	**0,8**	**15,7**	**27,0**	**37,0**	**45,0**	**55,0**
Average height of foam in cm	4,8	3,7	6,6	6,1	7,3	6,3

Table 2 shows the averages of the different temperatures. They were calculated using the formula above. It could be concluded that catalase does not work so well at lower temperatures (up to ~ 16°C), and better at higher temperatures, because for lower temperatures the foam did not reach up so high as for higher temperatures. However, the results are too similar to show a clear trend.



Graph 2

The results of ***Table 2*** are presented graphically in ***Graph 2***, where the outcomes can be seen and interpreted easier. As was obvious from the table already, catalase seems to work better for higher temperatures than for low ones. Nonetheless, since no clear trend can be seen this is more or less only a guess. What is strange about the outcomes is that they do not rise and fall steadily but rather peak and then fall and then peak again. This makes any interpretation of the results difficult.

However, it seems that at 45°C the enzyme works best because it is the temperature with the highest average (7,3 cm). In comparison, at 15,7°C, we measured the lowest average with only 3,7 cm.

It can also be seen at the graph that no value was recorded where the liver solution showed no, or only very little reaction with the H_2O_2 solution. This is certainly a drawback of our experiment since no proper conclusion can be reached on which actually is the optimum temperature (it is only known that enzymes prefer warmer temperatures) for the enzyme to work or at which temperature the enzyme denatures.

The average was calculated because the data alone did not show any trends and by finding the averages I hoped to be able to see a proper trend, which in the end apparently was not the case. The graph allows me to compare the results more easily.

Data processing and Presentation 2

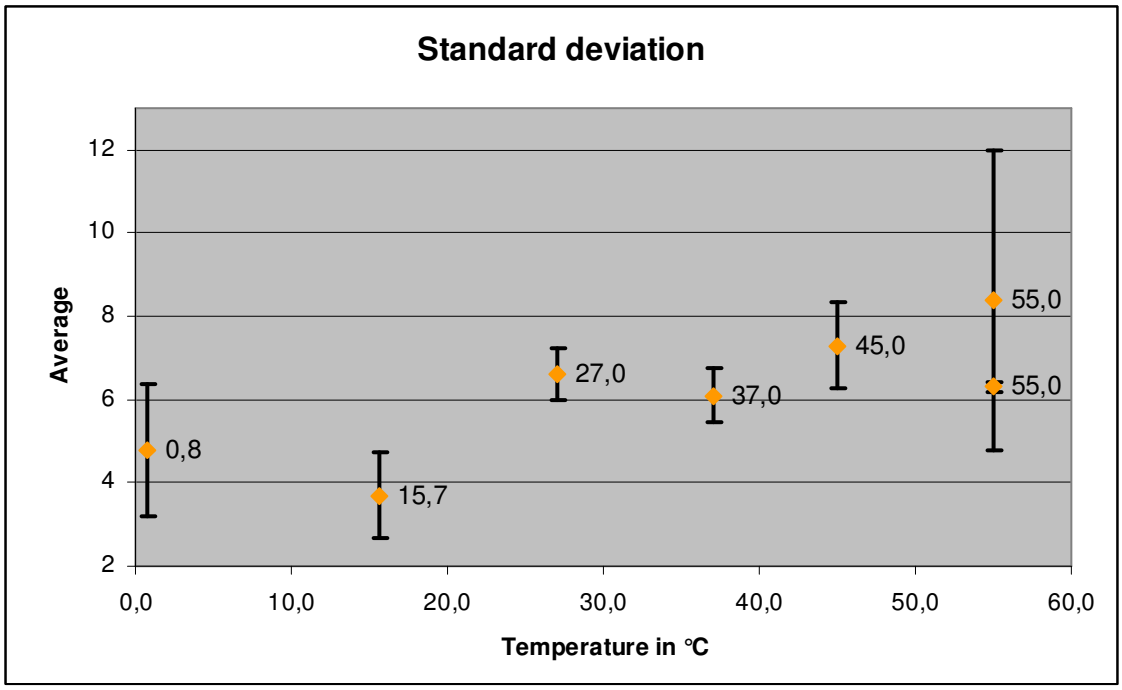
Next it would be interesting to find out the standard deviation of the values. This way we can see which values are more reliable and which ones are less reliable and therefore maybe we might be able to draw a conclusion on which temperature is best for catalase to work.

Using the values and the averages calculated above, we can easily calculate the standard deviation. The last column is calculated with 4 values, which means that 14,6 cm is included.

Table 3: averages and standard deviations of the different methods.

Temperature (°C)	**0,8**	**15,7**	**27,0**	**37,0**	**45,0**	**55,0**	**55,0**
Average height of foam in cm	4,8	3,7	6,6	6,1	7,3	6,3	8,4
Standard deviation	1,58	1,03	0,62	0,66	1,04	0,14	3,60

Table 3 shows the averages of the different temperatures as well as their standard deviations. The standard deviation for 55,0°C was calculated once including the value of 14,6 cm and once without it. This, obviously gave very different results. It can be seen that, not considering the probable measuring error, 55,0°C would be the best temperature for catalase to work, while 0,8°C would be the worst, because the values were not so accurate for it. If we however take the 14,6 cm-value into consideration, 55,0°C seems by far the worst temperature because the standard deviation is so high and therefore there are vast uncertainties.



Graph 3

The results of ***Table 3*** are presented graphically in ***Graph 3***, the yellow point showing the average and the black lines represent the standard deviation. Similarly to what we expected from the table, the value at 55°C has least standard deviation when calculated without the value of 14,6 cm, while on the other hand it has the highest standard deviation when calculated with 14,6 cm. However, it is difficult to draw a conclusion out of this because the average, for instance for 45,0°C is higher, but on the other hand also the standard deviation is higher which means that the results were not so accurate. However, since the higher values (from 27,0°C onwards) have a lower standard deviation and a high average, it can maybe be concluded that the higher temperatures offer better conditions for catalase to work.

Conclusion and evaluation

Due to the fact that the results of the various measurements are so similar, it is not really to draw a proper, representative conclusion. Nonetheless, according to the averages as well as the standard deviations it seems that warmer temperatures aid the functioning of the enzyme more than cooler ones. However, if we consider the individual values, no real trend can be found out. The only thing worth mentioning is the 3rd test tube for 55°C, which shows an unnaturally high value. Therefore, we can say that this value was a measuring error since it is more than double as high as any of the other vales. The averages and standard deviations are therefore probably most representative when they are calculated excluding this value, which I did.

It can be neither said at which exact temperature the enzyme works best, because there is no clear peak in the graph, nor at which temperature the enzyme does not work yet or at which temperature it starts to denaturate.

The reason why these measurements are so similar to each other could be the fact that we did not wait until the foam had reached his maximum height but we simply measured the height after a certain time interval. We did therefore not consider that the foam could maybe still rise. If we would have waited longer, the results might have been more representative. Nonetheless, it can probably still be said that the enzyme does not work so well at lower temperatures and that it still does not denature at 55°C because then the foam would not have risen so much. Consequently it is obvious that it takes significantly higher temperatures for catalase to denature.

Evaluating procedure and results

In general, it can be said that the results of our experiment are not very reliable because we did a number of mistakes. This is also the reason why not proper solution can be drawn. Nonetheless, the fact that at lower temperatures catalase did not work so well, is probably also true when the experiment is done better. The temperature at which catalase works best could only be found out by another experiment, because we did not leave enough time for the foam the catalase produced to develop fully.
The reasons why at lower temperatures catalase might not works so well are because it is used to body temperature and every enzyme has a preferred temperature at which it works best. However, in this connection it seems strange that at a temperature of 55°C, which is significantly higher than body temperature, the catalase does not start denaturing yet.
One big problem was getting to the exact temperatures, and as the water cooker did not work properly, it was difficult to reach temperatures higher than 55°C. Also, maybe because the water was not completely pure, it was not possible to reach exactly 0°C. With better equipment and more time for preparation, these errors could have been avoided.

We encountered the following problems in our investigation:

1. The range of temperatures was too close apart to really find out which temperature influenced the development of the foam.
2. Sometimes the foam developed even higher after we had done the measurement giving an inaccurate result.
3. Sometimes not the whole amount of H_2O_2 reached the liver solution because it touched the walls of the test tube, which surely also influenced the development of the foam.
4. The carrying out of the experiment was sometimes not very accurate, on the one hand because of the H2O2 problem explained below, on the other hand because we used a ruler for measuring which is somehow inaccurate.
5. In many cases, the results for one temperature were quite far apart so it was difficult to say which one is most meaningful for this temperature.

Improving the investigation

The investigation could be improved by:

1. Using even higher temperatures and more different temperatures in order to get a better and more representative curve and find out at which temperature catalase really starts to denature.
2. Waiting until the foam had reached its maximum height and only then marking the height and finally measuring it, because this gives the real reaction rate of the enzyme, or at least coming to an agreement on when we should measure the foam – when it is at its highest point or

after a certain amount of time? The best method has to be found out beforehand, but it probably is the simply record when the foam is at its highest place.

3. Trying to put the drops of H_2O_2 really directly on the liver solution. This could be reached by putting the pipette directly over the liver solution before squeezing the drops in.
4. Being more accurate throughout the whole experiment and using another device for measuring the height of the foam such as a measuring tape. It is also recommendable that the height of the foam is first marked with a pen because this way you do not have to measure the height in a rush and the results are therefore also more exact.
5. Repeating the experiment more often (with more test tubes) to have more results and then calculating the average to overcome measuring or recording errors, obtain a reliable result and see a clearer trend.

Further possible improvement:

6. Trying the experiment with different amounts of H_2O_2 solution and liver solution so that any assumptions can be further proven.