

Detecting sugar: an everyday problem when facing diabetes

Fred Engelbrecht and **Thomas Wendt** from the ExploHeidelberg Teaching Lab describe some experiments on sugar detection to demonstrate the problems that people with diabetes face every day.



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Diabetes, a disease of modern civilisation

The monosaccharide glucose is the most important source of energy in the living eukaryotic organism and is used by cells in aerobic or anaerobic respiration. It also serves as a precursor in the production of proteins and in lipid metabolism. Therefore, it is a central molecule in several metabolic pathways and its concentration in the bloodstream must be tightly regulated by insulin and glucagon.

Diabetes mellitus (or simply **diabetes**) is a syndrome characterised by disordered glucose metabolism and overly high blood sugar levels (hyperglycaemia). This is due either to low levels of the hormone insulin or to an abnormal resistance to the effect of insulin coupled with levels of insulin secretion that are too low to compensate for the resistance.

There are two major forms of diabetes: Type 1 and Type 2. Although they have different causes, patients with either form are unable to produce sufficient insulin in the beta cells of the pancreas to prevent hyperglycaemia.

Type 1 diabetes comprises about 10% of all diabetes cases in Europe, and is characterised by the loss of the pancreatic beta cells, usually by autoimmune destruction. Since Type 1 diabetes often affects patients at a young age, it is also named juvenile diabetes. It is the more severe form of the disease because there is no treatment. Instead, patients must adjust their lifestyles, for example by improving their diet, taking regular exercise and monitoring their blood sugar levels. Additionally, subcutaneous injections or the continuous delivery of insulin by a pump into the blood circulation system are necessary to avoid coma or death.

Type 2 diabetes is due to insulin resistance or reduced insulin sensitivity in the target tissues, combined with insufficient insulin secretion. The low-



ExploHeidelberg

The ExploHeidelberg^{w3} is an informal learning and interactive science centre. It consists of three different components: an interactive exhibition, a media lab and a biotechnology teaching lab.

In the interactive exhibition, about 50 exhibits challenge visitors to experience optical, acoustic and mechanical phenomena. The pedagogical concepts and design of the interactive exhibition are developed in close collaboration with the University of Education Heidelberg^{w4}. Trainee teachers and staff members from the physics department are involved as exhibition scouts, and in designing exhibits and developing workshops, teacher training and research projects.

The teaching lab offers middle- and high-school students the opportunity to perform biotechnology experiments in full-day practical courses that are not possible in the classroom.

A media lab with 12 workstations, webcast facilities and a video imaging workstation complements the study centre.

The interactive exhibition and the media lab are open daily to the general public and focus on interesting the visitor in life sciences in general. The teaching lab offers school and university students, teachers and trainee teachers special courses related to the school curriculum, giving an insight into modern biotechnology techniques. Participants can choose from one-day courses on handling DNA or proteins and specialised one-week courses involving sophisticated techniques that are usually only taught at university level.

BACKGROUND

ered response of the body tissues to insulin almost certainly involves the insulin receptors in cell membranes. This causes the body to need abnormally high amounts of insulin to maintain normal blood sugar levels, and diabetes develops when the beta cells cannot meet this demand. Type 2 diabetes, commonly known as adult-onset diabetes, usually appears after the age of 30. In most cases, it is connected with obesity and too little physical exercise; changing to a healthier lifestyle can improve the condition or in some cases even cure it. See Dugi (2006) for more details of diabetes.

People affected by either type of diabetes need to learn how to live

with the symptoms of the disease. These include frequent urination, increased thirst and, consequently, increased fluid intake. Since large numbers of children are affected by diabetes, it is essential to teach students about the disease from an early stage. Diabetes sufferers need to learn how to minimise their symptoms or even prevent the disease by eating a healthy diet and taking enough exercise. Healthy children should understand the needs of their affected friends.

We have therefore put together some experiments to enable students to detect carbohydrates. One series of experiments detects whether or not a solution contains starch, proteins, or sugars such as glucose, lactose or

Image courtesy of Thomas Wendt



Fehling's reaction (left), Lugol's reaction (middle), protein assay (right)

sucrose. Once the sugars are identified, further experiments determine, using an enzymatic reaction, which samples contain lactose or glucose. The principle of these experiments is the same as in assays to determine blood glucose levels for the diagnosis of diabetes, or to measure glucose and/or lactose levels, for example in fruit juices, milk and dairy products. These experiments, therefore, give students an idea of how diabetes sufferers can monitor their sugar status.

Experiment 1: Detection of sugar, starch and protein

Students receive five samples, labelled A to E, which contain starch, protein (bovine serum albumin), the monosaccharide glucose, or the disaccharides lactose or sucrose. All solutions are at a concentration of 0.1% in water. You could also test samples of colourless glucose-containing energy drinks (e.g. Powerade®-Lemon). Using different reagent solutions, students should determine which of the five samples contain sugar, starch or protein.

For a class of 30 students working in pairs, you will need the following solutions:

- Fresh Fehling's solution, made by mixing equal volumes of light blue Fehling's I solution (7 g $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ dissolved in 100 ml distilled water) and colourless Fehling's II solution (35 g $\text{C}_4\text{H}_4\text{KNaO}_6 \cdot 4 \text{H}_2\text{O}$ and 10 g NaOH dissolved in 100 ml distilled water). The solution should be mixed shortly before it is needed.
- Lugol's solution, made by dissolving 1 g iodine (I_2) and 2 g potassi-

um iodide (KI) in 50 ml distilled water.

- Coomassie Brilliant Blue dye for the Bradford assay is commercially available, for example from Biorad^{w1}.

a) Detection of a reducing sugar (Fehling's reaction)

1. Pipette a 1 ml sample of solutions A to E into each of five different reaction tubes and heat the contents to 60 °C in a water bath for 2 min.
2. Add 16 µl of the deep blue fresh Fehling's solution to each reaction tube and incubate the tubes at 60 °C for a further 10 min, or until a colour reaction is observed and a precipitate forms.

The solutions containing reducing sugars like fructose, glucose or lactose should turn red and develop a red precipitate (the dissolved copper (II) ions are reduced to insoluble copper (I) oxide), whereas there should be no colour change with sucrose or starch. The protein solution should turn pale violet.

b) Starch detection (Lugol's solution)

1. Pipette a 500 µl sample of solutions A to E into each of five new reaction tubes.
2. Add 50 µl of Lugol's solution to each tube and observe the colour reaction.

Lugol's solution is an indicator to test for starch. The dye will stain starch as it interacts with the coiled structure of the polysaccharide, giving rise to a deep blue colour. It will not react with monosaccharides (glucose) or disaccharides (lactose or sucrose).

c) Protein detection

The protein assay is based on the Bradford dye binding procedure that measures the colour change of the Coomassie Brilliant Blue dye when it binds to protein.

1. Pipette a 20 µl sample of solutions A to E into each of five new reaction tubes, then add 800 µl deionised water and 200 µl Coomassie Brilliant Blue dye (Bradford reagent).

Table 1: Example of results obtained in Experiment 1

	Fehling's reaction	Lugol's reaction	Protein assay	Compound
Solution A	Red precipitate	Brown	Brown	Reducing Sugar
Solution B	Blue solution	Dark blue	Brown	Starch
Solution C	Red precipitate	Brown	Brown	Reducing Sugar
Solution D	Violet solution	Brown	Blue	Protein
Solution E	Blue solution	Brown	Brown	Sucrose

2. Mix the reagents, leave the reaction for 5 min, and observe the colour reaction.

In the presence of protein, the solution will turn blue (this can be measured in a photometer at 595 nm). The samples containing sugar or starch will not change colour.

Safety note: The Biorad protein assay solution contains methanol and phosphoric acid and should, therefore, be used with caution.

Results and interpretation

Solution B gives a positive reaction with Lugol's solution, thus revealing it to contain starch. Solution D gives a positive result with the Bradford assay, revealing itself to be a protein solution. Solutions A and C give a red precipitate during the Fehling's reaction,

and can therefore be identified as the reducing sugar samples glucose and lactose (although it is not possible at this stage to tell which is which). The remaining solution, E, shows no reaction in any of the tests and must therefore be the sucrose solution.

Experiment 2: Enzymatic determination of sugar

In this second experiment, the two remaining solutions A and C are tested again, to see which of them contains lactose and which glucose. For this experiment we use a commercially available kit, EnzyPlus EZS 962+ lactose/D-glucose, which can be purchased from BioControl^{w2}. The procedure is similar to that routinely used by diabetes patients to monitor their blood glucose levels. The standard

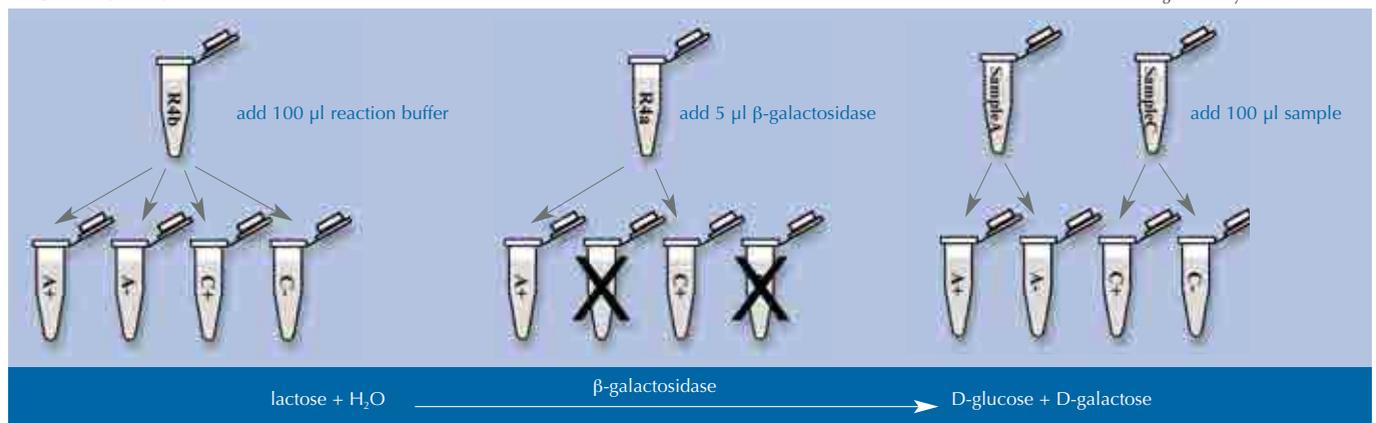
protocol for the product has been modified and downscaled, so that a larger number of experiments can be performed with the reagents provided. One kit provides enough materials for 20 pairs of students.

The principle of the test is as follows (see figure below):

- The disaccharide lactose is hydrolysed by the enzyme β -galactosidase to D-galactose and D-glucose (Step 1 in the diagram below).
- In the presence of ATP, D-glucose is specifically phosphorylated by the hexokinase to glucose-6-phosphate; simultaneously, adenosine-5'-diphosphate (ADP) is formed (Step 2).
- The glucose-6-phosphate is oxidised by the glucose-6-phosphate dehydrogenase to gluconate-6-phosphate.

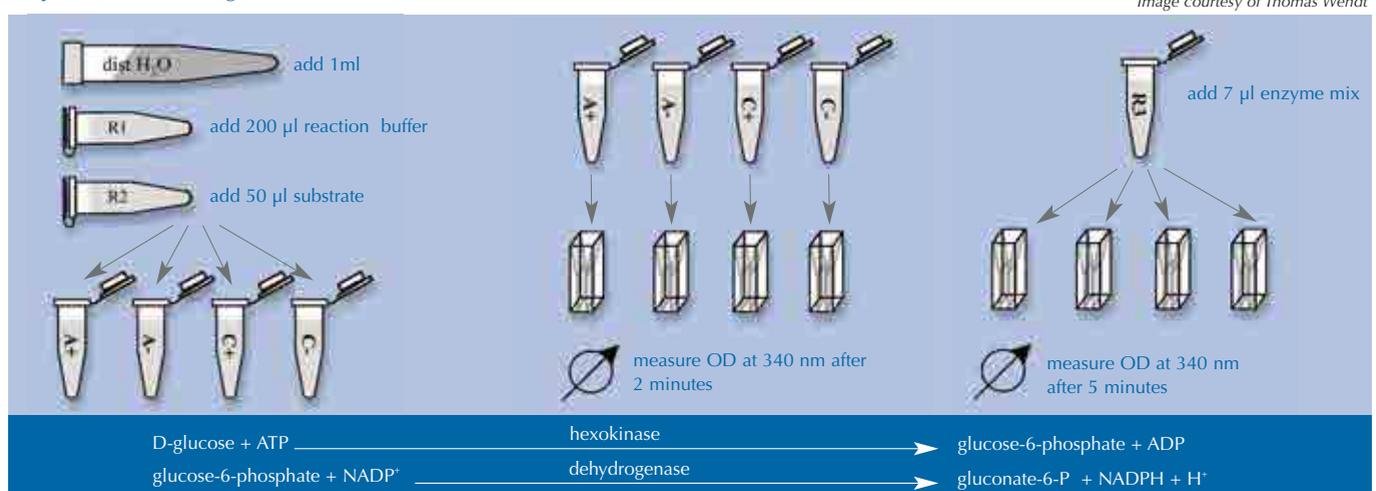
Step 1: Hydrolysis of lactose

Image courtesy of Thomas Wendt



Step 2: Detection of glucose

Image courtesy of Thomas Wendt



- During this reaction, NADP^+ is reduced to NADPH. The amount of NADPH formed in this reaction is stoichiometric to the amount of lactose and can be measured photometrically by the increase in absorbance at 340 nm.

To perform the reaction:

1. Take four 1.5 ml reaction tubes and label them A+, A-, C+ and C-. Put 100 μl reagent buffer R4b (phosphate buffer pH 6.6) into each tube, and add 5 μl β -galac-

tosidase solution R4a into tubes A+ and C+ (but not tubes A- or C-).

2. Add 100 μl of solution A to tubes A+ and A- and 100 μl of solution C to the tubes labelled C+ and C-.

Note: In the remaining steps of this experiment, all four samples are treated identically.

3. Leave all samples for 30 min at 37 °C in a water bath while lactose hydrolysis occurs.
4. After the incubation, add 1 ml distilled water, 200 μl reaction buffer R1 and 50 μl reagent R2 (contain-

ing ATP and NADP, respectively) to all four reaction tubes and mix thoroughly.

5. Transfer 1 ml of each reaction mixture into separate photometer cuvettes and measure the optical density at 340 nm (OD_{340}) after 2 min.
6. To each cuvette, add 7 μl enzyme mix R3 containing the hexokinase and the glucose-6-phosphate-dehydrogenase, incubate for a further 5 min, and measure the absorption at 340 nm again.

Table 2: Example of results obtained in Experiment 2

	First measurement (OD_{340})	Second measurement (OD_{340})	Result
Sample A +	0.09	2.43	} Glucose
Sample A -	0.09	2.37	
Sample C +	0.10	1.43	} Lactose
Sample C -	0.09	0.10	



Image courtesy of iStockphoto

Results and interpretation

All four samples should give low absorption values when first measured, indicating the absence of NADPH. The second measurement (after addition of the enzymes) should allow solutions A and C to be distinguished. Since solution A gives positive results independently of whether or not β -galactosidase had been added, it can be concluded that it contains glucose. In contrast, a change in the absorption of solution C should only be measured in the presence of β -galactosidase (C+), indicating that it contains lactose.

During the practical, participants get basic information on diabetes and the problems that patients with diabetes face. To monitor their blood glucose level, patients use a test-strip glucose-monitoring system that acts like a black box but is based on the

principle used in Experiment 2. Performing such experiments may raise awareness of diabetes and of how the disease can be survived or prevented through lifestyle changes.

The experiments described here can be safely performed in normal school laboratories, since the reagents used are not hazardous materials in the sense of the Hazardous Substances Regulations (EC Regulation 67/548/EEC). The level of sodium azide, the preservative in the reagents, is below the lowest level of toxicity for a preparation, according to the Directive 1999/45/EC.

Web references

w1 – Biorad: www.bio-rad.com

w2 – BioControl:
www.rapidmethods.com

w3 – ExploHeidelberg:
www.explo-heidelberg.de

w4 – University of Education
Heidelberg: www.ph-heidelberg.de



References

- Dugi K (2006) Diabetes mellitus.
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www.scienceinschool.org/2006/issue1/diabetes/



In this article, Fred Engelbrecht and Thomas Wendt address the important topic of diabetes and propose two experiments to give an insight into the main components of food and into the science behind glucose test strips commonly used by diabetes patients.

The style of the text, clear and appropriate, is easier in the first part (about diabetes); the experiments are a bit more challenging, giving teachers the opportunity to use the different sections at different secondary-school levels.

The article can be used in the biology, chemistry and health education curricula, with the possibility of an interdisciplinary approach to the issues related to diabetes, for example linking biochemistry, biology and health education. Given the widespread nature of this disease, the text is useful as a starting point to promote active citizenship and full social inclusion of diabetic students.

The article could be used to address the topics of carbohydrates and proteins; metabolism (biochemistry); the digestive system (biology); food and health (health education); and laboratory training (chemistry).

The first part can be used to test the comprehension of diabetes, the second part to test the comprehension of technical issues. Example questions include:

- Which of the following features is not typical of diabetes mellitus?
 - High blood glucose level
 - Insufficient insulin production or resistance to the effect of insulin
 - Low blood glucose level
 - Frequent urination and increased thirst.
- The data shown in Table 2 mean that:
 - Glucose needs β -galactosidase to produce NADPH
 - Lactose needs β -galactosidase to produce NADPH
 - Lactose doesn't need β -galactosidase to produce NADPH
 - Glucose needs glucose-6-phosphate dehydrogenase to produce NADP⁺.

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