

H₂-BREATH TESTS FOR MEDICAL RESEARCH AND CLINICAL DIAGNOSIS

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1. INTRODUCTION

While the lowest layer of the terrestrial atmosphere, the troposphere, contains 0.575 ppm hydrogen (H₂), breath exhaled by healthy people preferentially holds 20 to 30 ppm or even more. (Deviations from this rule are at least partially due to the fact that some people hold more methane than hydrogen producers in their intestinal micro-fauna. These people exhale preferentially methane instead of hydrogen.) The enhanced hydrogen expiration goes back to the fact that part of the ingested carbohydrates (and proteins) is not absorbed or digested and absorbed by the intestinal mucosa, but is fermented to form hydrogen (or methane) by bacteria settling either in the colon or, especially in case of a bacterial overgrowth, also in the small intestine. Part of this hydrogen is dissolved in mucosal blood, transported to the lungs within a few minutes and finally appears in breath. Thus an orally ingested carbohydrate (D-glucose, D-fructose, D-galactose, D-xylose; D-lactose, D-sucrose, D-trehalose, D-lactulose; starch etc.) or a substance similar to carbohydrates with respect to its molecular structure (sorbitol, xylitol, lactitol, mannitol etc.) causes an increased hydrogen concentration in breath, either because the substance is not or only partially absorbed in the small intestine and therefore arrives at the colon, where it is fermented by anaerobic bacteria settling in the colon, or because the ingested substance is already fermented to form H₂ in the small intestine because of an overgrowth with H₂ producing anaerobic bacteria in this very part of the intestine.

Plots of H₂-concentration in breath over time after ingestion of a carbohydrate or any other test substance may therefore have the following shapes:

1.) No ascent of H₂-concentrations over more than three hours

Reasons: The substrate is not fermented by H₂ producing bacteria in the entire intestine

Inferences for diagnosis: No H₂ producing bacteria settling in the entire intestinal system or the test substance is absorbed in the intestinal mucosa at a rate large in comparison to the rate of bacterial fermentation

2.) H₂-concentration passes a maximum more than one hour after ingestion of the test substance and then approximates the time axis again.

Reasons: The large intestine is settled by anaerobic bacteria fermenting at least part of the substrate to form H₂.

Inferences for diagnosis: Diseases which shorten or prolong residence time of the substrate in the small intestine in comparison to normal values between one or a few hours.

3.) H₂-concentration passes a maximum during the first hour after ingestion of the test substance and then approximates the time axis again.

Reasons: All the substrate is either absorbed and fermented or only fermented to form H₂ in the small intestine. The test substance therefore does not arrive at the large intestine.

Inferences for diagnosis: Bacterial overgrowth of the small intestine

4.) H₂-concentration passes a first maximum during the first hour after the ingestion of the substrate, a second one after more than one hour and then approximates the time axis again.

Reasons: A part of the test substance is fermented in the small intestine, another in the large intestine.

Inferences for diagnosis: Bacterial overgrowth of the small intestine and, should the occasion arise, also diseases bringing about abnormal residence times in the small intestine.

Thus by means of H₂-breath tests intestinal disorders of the following kind can be diagnosed:

- inflammatory intestinal diseases [lactulose-H₂ breath test]
- abnormal residence time of carbohydrates in the gastrointestinal system (orocoecal residence time) [lactulose-H₂ breath test]
- Evaluation of therapeutic efficiency of therapeutics against myeloid leukaemia [lactulose-H₂ breath test, lactulose-mannitol-H₂ breath test]
- coeliac disease [lactulose-H₂ breath test, D-xylose-H₂ breath test]
- Bacterial overgrowth in certain intestinal sections, diverticula [D-xylose-H₂ breath tests, D-glucose-H₂ breath test]
- Malabsorption or maldigestion, respectively, of certain carbohydrates in the intestinal system
- Lactose intolerance [lactose-H₂ breath test]
- Sucrose intolerance [sucrose-H₂ breath test]

2. PROCEDURE OF H₂-BREATH TESTS

H₂-breath tests should not be performed less than four weeks after therapy with antibiotics, bowel lavage or enteroscopy. Before taking up the test meal with the substrate patients have to fast at least for six hours. During this time and during breath sampling chewing gums must not be used because they usually contain sorbitol which may be fermented to form hydrogen in the intestinal system. Starting at least 30 minutes before the ingestion of the substrate the individuals must abstain from smoking and physical strain.

Alveolar air, i.e. the last part of one single expiratory action (about 150 ml), has to be collected immediately before and then in certain intervals for several hours after the intake of the test meal. Breath sampling rhythms, as well as the amounts of substrates to be administered, are indicated in paragraphs 3 or 5 of this monograph, respectively. Evaluation of the measured data is based on the course of H₂-concentrations in dependence on time (in comparison with the H₂-concentration in the inhaled air). Usually the so-called cut-off value Δ , i.e. the difference of the maximum H₂-concentration in breath a certain time after substrate intake minus the H₂-concentration immediately before substrate intake, is used as the diagnostic criterion. If this difference exceeds this critical value Δ , a certain metabolic anomaly must be assumed. Since the gastric residence times of any substrate scatter in a wide range even in normals and H₂-production usually does not begin before the substrate has passed stomach and duodenum, breath sampling must not be finished, before the H₂-concentration in breath has passed its maximum.

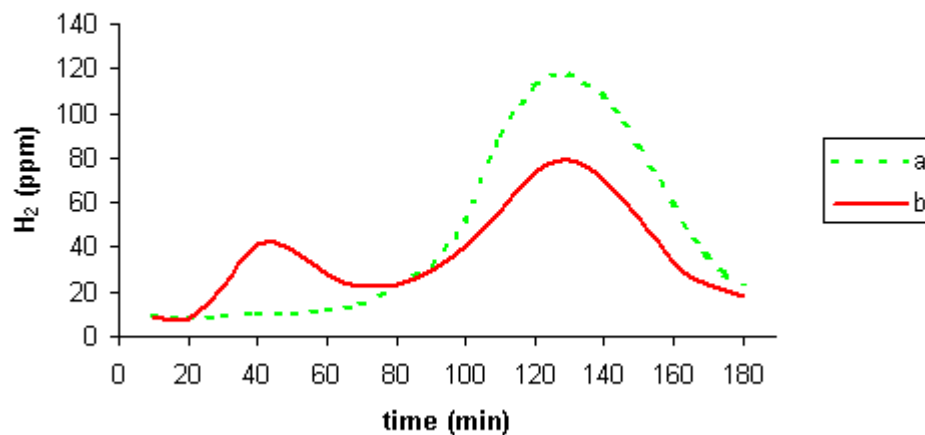
3. DESCRIPTION OF SEVERAL IMPORTANT H₂-BREATH TESTS

In the following some H₂-breath tests nowadays especially important are described in detail.

1.) Lactulose-H₂-Breath Test

Lactulose is an artificially synthesised disaccharide composed of fructose and galactose, for which an enzyme splitting it into the corresponding monosaccharides does not exist. Plots of H₂-concentration in breath over time after ingestion of lactulose may therefore have the following shapes:

Figure 1: Lactulose-H₂-Breath Test



a) Normal.

For want of a suitable enzyme lactulose is not split into the monosaccharides. Bacterial overgrowth in the small intestine does not exist. At the end of the oro-coecal transit time the test substance therefore arrives at the large intestine, where it is fermented to form hydrogen (or methane). Hydrogen (or methane, respectively) is absorbed through the intestinal wall and then dissolved into the bloodstream, released into the lungs through the capillary blood vessels surrounding the alveoli, and finally expired.

b) Pathologic.

Because of bacterial overgrowth of the small intestine fermentation and production of hydrogen already starts in the small intestine.

The lactulose-H₂-breath test is the most widespread non-invasive test for determining oro-coecal transit time. It serves to investigate the residence time of certain food constituents, particularly carbohydrates, in the intestinal system. With respect to selectivity and specificity, the test for assessing oro-coecal transit time is surpassed by the lactulose-[¹³C, ¹⁵N]ureide breath test, but the latter is more expensive. (Therefore the following procedure is usual in medical diagnosis: If the results of both tests coincide, then the result is assumed to be true. If the two tests yield contradictory oro-coecal transit times, however, the decision must be forced by an invasive method.) Very early ascent of H₂-concentration in breath points to bacterial overgrowth in the small intestine, retarded H₂-expiration indicates prolonged transit time in this part of the intestine. Diagnosis of cystic fibrosis, hyperglycaemia, gastrooesophagic reflux and coeliac disease are further, emerging diagnostic objectives of the lactulose-H₂-breath test. In addition the lactulose-H₂-breath test is applied for evaluating the therapeutic efficiency of medicaments against myeloid leukaemia and, in view of the negative effects of HIV disease on lactose absorption, also against HIV disease.

If the H₂-content of breath increases by more than 20 ppm after having taken up 20g lactulose, then carbohydrate malabsorption must be assumed.

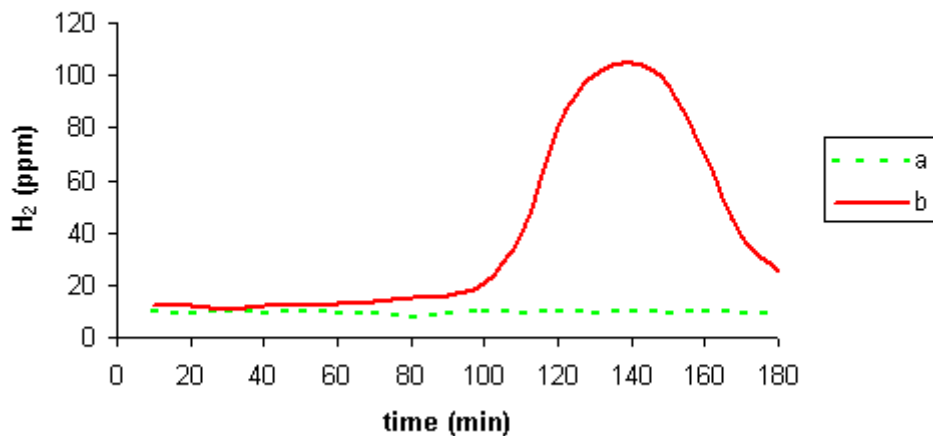
Implementation of the lactulose-H₂-breath test:

Children up to an age of six months ingest 3.34 g lactulose in isotonic solution (3.34 g/5 ml Duphar-Duphalaco-syrup), children older than six months 6.68 g lactulose/10 ml syrup. Adults are supplied with 10 g lactulose dissolved in 150 ml of water. Breath samples are taken immediately before and 30, 60, 120 and 150 minutes after substrate ingestion. For diagnosing asymptomatic diabetes mellitus the lactulose-H₂-breath test is obviously not suitable.

2.) Lactose-H₂-Breath Test

Lactose is a disaccharide. The enzyme lactase splits it to form galactose and glucose, which are absorbed in the small intestine. Plots of H₂-concentration in breath over time after ingestion of lactose may therefore have the following shapes:

Figure 2: Lactose-H₂-Breath Test



a) Normal.

Lactose is split into galactose and glucose and then absorbed in the small intestine. In normals fermentation by anaerobic microorganisms therefore proceeds either not until the large intestine or not at all.

b) Pathologic

(lactose malabsorption, lactose intolerance). For want of lactase the test substance is neither split into the monosaccharides nor absorbed in the small intestine. Therefore the substrate is fermented to form hydrogen not until the large intestine. There this hydrogen is absorbed through the intestinal wall and then dissolved into the bloodstream, released into the lungs through the capillary blood vessels surrounding the alveoli and finally expired.

The lactose-H₂-breath test is used for the diagnosis of lactose malabsorption, which is highly abundant all over the world. The test is also applied to control the therapy of lactose intolerance by loperamide.

Implementation of the lactose-H₂-breath test:

For diagnosing lactase deficiency 2g/kg (maximum 20g) lactose, dissolved in 100 ml of water, are orally administered after an overnight fast. Breath samples are taken immediately before and 30, 60, 120 and 150 minutes after substrate ingestion. We recommend a cut-off value of $\Delta = 30$ ppm for distinguishing patients with lactase deficiency and normals.

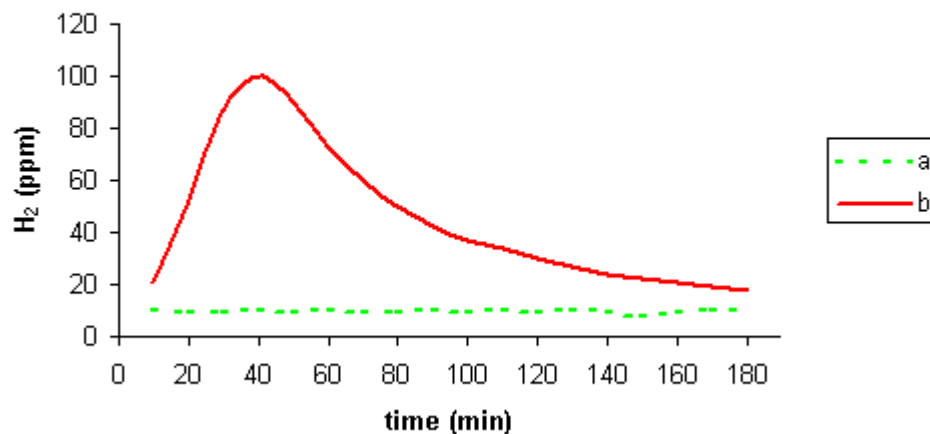
Sensitivity and specificity are then in the range of 90 to 96%. If additional symptoms like abdominal pain are reported, lactose intolerance may be assumed. Starting 24 hours before

substrate uptake and during the test beans, pies, larger amounts of apples, onions, cabbage, bread fresh from the oven and other heavy food must not be taken in.

3.) The Glucose-H₂-Breath Test

Glucose is a monosaccharide rapidly and completely absorbed in the small intestine. In case of bacterial overgrowth in the small intestine absorption and bacterial fermentation compete with one another. Plots of H₂-concentration in breath over time after ingestion of glucose may therefore have the following shapes:

Figure 3: Glucose-H₂-Breath Test



a) Normal.

Glucose is rapidly and completely absorbed in the small intestine and therefore does not arrive at the large intestine

b) Pathologic.

Bacterial overgrowth of the small intestine. There and only there absorption and bacterial fermentation combined with hydrogen production compete with one another.

The glucose-H₂-breath test is applied for diagnosing bacterial overgrowth of the small intestine, in combination with galactose-H₂-breath test also for the diagnosis of glucose/galactose malabsorption. The test is also considered for the diagnosis of exocrine pancreas insufficiency.

Implementation of the glucose-H₂-breath test:

After an overnight fast 75 g glucose, dissolved in 400 ml of water, is orally administered. Breath is sampled immediately before and 45, 90, 135 and 180 minutes after substrate intake. If H₂-concentration ascends by more than $\Delta = 20$ ppm, then bacterial overgrowth of the small intestine must be assumed. Sensitivity and specificity of the glucose-H₂-breath test, however, are only in the order of 60 to 90% or 75 to 100%, respectively. Nevertheless the test went its way for want of better alternatives.

4.) The Fructose-H₂-Breath Test

The fructose-H₂-breath test is used for the diagnosis of fructose-malabsorption. The test is absolutely contraindicated for patients with hereditary fructose intolerance. In this case the application of this diagnostic tool would be life-threatening. (The abundance of this metabolic anomaly is of the order of 1:10 000 to 1:15 000. Symptoms are a hyperglycaemic shock after intake of food containing fructose (fruits, milk products containing saccharose, sweets etc.) in infancy, metabolic disorders accompanied by vomiting, fever and dystrophy, hepatomegalia

and hepatic malfunctions. Diagnosis is possible on the basis of a deficiency of the subunit of the enzyme aldolase in the liver or the small intestinal mucosa.)

Implementation of the fructose-H₂-breath test:

After a nocturnal fasting period 25 g of fructose, dissolved in 250 ml of water, are orally administered. Breath samples are taken immediately before and 45, 90, 135 and 180 minutes after substrate intake. If H₂-concentration ascends by more than $\Delta = 20$ ppm, then fructose malabsorption must be assumed.

5.) The Xylose-H₂-Breath Test

The D-xylose-H₂-breath test is used for diagnosing intestinal absorption deficiencies, e.g. coeliac disease. In normals D-xylose is (less rapidly than glucose and galactose) absorbed in duodenum and upper jejunum. Plots of H₂-concentration in breath over time after ingestion of D-xylose therefore have shapes similar to those shown in figure 2. Unlike glucose-H₂-breath test the test does not discriminate between bacterial overgrowth and malabsorption in the small intestine.

Implementation of the xylose-H₂-breath test:

For determining xylose absorption 25 g D-xylose in aqueous solution are administered. The faster the substrate is absorbed, the smaller is the amount of the substrate, which arrives at the colon and can be fermented there to form hydrogen. Breath is sampled immediately before and 45, 90, 135 and 180 minutes after substrate intake. The cut-off-value for distinguishing patients with intestinal absorption deficiencies from normals is $\Delta = 20$ ppm.

6.) The Sucrose-H₂-Breath Test

The sucrose-H₂-breath test is used for diagnosing sucrose intolerance and sucrose-isomaltose (starch)-malabsorption. Sucrose is split to form glucose and fructose in the small intestine. In normals these monosaccharides are completely absorbed in this part of the bowel without attaining at the large intestine. In case of bacterial overgrowth in the small intestine hydrogen production by fermentation competes with absorption and metabolisation. Plots of H₂-concentration in breath over time after ingestion of sucrose may therefore have shapes similar to those shown in figure 2.

Implementation of the sucrose-H₂-breath test:

For diagnosing sucrose intolerance after overnight fast 2g/kg (maximum 20g) sucrose, dissolved in 100 ml of water, are orally administered. Breath is sampled immediately before and 30, 60, 120, and 150 minutes after substrate intake. The cut-off value for distinguishing patients with sucrose intolerance from normals is $\Delta = 20$ ppm.

7.) The Sorbitol-H₂-Breath Test

For the diagnosis of coeliac disease occasionally also the H₂-exhalation with breath after intake of the hexavalent alcohol sorbitol can be measured. Sorbitol is absorbed in the small intestine. Plots of H₂-concentration in breath over time after ingestion of sorbitol may therefore have shapes similar to those shown in figure 2.

Implementation of the sorbitol-H₂-breath test:

After a 12-hour fasting period 5 g sorbitol, dissolved in 250 ml of distilled water, are administered orally. During the fasting period and the test itself the individuals take only tea, coffee or tap water and refrain from higher physical activity. FAN recommends breath sampling immediately before and 30, 60, 120, 180 and 240 minutes after substrate intake and to use a cut-off value of $\Delta = 10$ ppm for distinguishing coeliac disease patients from normals.

4. MEASURING H₂-CONCENTRATIONS IN BREATH

For measuring H₂-concentrations in breath either thermal conductivity cells or hydrogen electrodes are used. Fischer ANalysen Instrumente GmbH, Leipzig, offers the H₂-monitor LactoFAN for this purpose. This hand-held device is based on an electrochemical fuel cell which works through the reaction of molecular hydrogen with an electrolyte at one electrode and molecular oxygen from ambient air at the other. This chemical reaction generates an electrical current proportional to H₂-concentration at the H₂-electrode read by a microprocessor, which detects H₂-concentrations in the expired gas and displays them in parts per million (ppm) with an accuracy of ± 1 ppm. LactoFAN is small (170 x 60 x 26 mm), light (175 g) and user-friendly. An autoreset function adjusts measured H₂-concentrations after ingestion of the test substance to values measured during fasting period. Fischer ANalysen Instrumente GmbH also offers accessories for taking, transport and storing breath samples, including sampling devices suitable for newborns, prematures and babies. Optionally an interface for PC controlling and data processing by means of special FANci software can be delivered.

5. SUMMARY

So far H₂-breath tests are preferentially applied in clinical research, the duration of the period of sampling the exhaled breath and the number of samples to be taken and measured does not matter. Sometimes breath is sampled for many hours at intervals of a few minutes. In clinical practice such longwinded and time-consuming procedures are less attractive. We therefore present simplified procedures for a series of important H₂-breath tests which we suppose to manage with shorter periods of sampling and/or smaller numbers of breath samples without considerable loss of sensitivity and specificity of the test. The last column of the following table presents cut off-values, i.e. the minimum ascents of H₂-concentration in breath after ingestion of test substance in comparison to the H₂-concentration in breath when fasting, which indicate pathologic metabolic situations.

Substrate	Amount	Times of Breath Sampling [min]	H ₂ -Ascent [ppm]
Lactulose	3.34 g in isotonic soltn. (< 6 months)	0, 30, 60, 120, 150	20
	6.68 g in isotonic soltn. (> 6 months)	0, 30, 60, 120, 150	20
	10 g in 150 ml of water (adults)	0, 30, 60, 120, 150	20
Lactose	2 g/kg (maximum amount 20 g) in 100 ml of water	0, 30, 60, 120, 150	30
D-Xylose	25 g in aqueous solution	0, 45, 90, 135, 180	20
Saccharose	2 g/kg in 100 ml of water (maximum amount 20 g)	0, 30, 60, 120, 150	20
Glucose	75 g in 400 ml of water	0, 45, 90, 135, 180	20
Fructose)*	25 g in 250 ml of water	0, 45, 90, 135, 180	20
Sorbitol	5 g in 250 ml of water	0, 30, 60, 120 180, 240	10

)* The test is absolutely contraindicated for patients with inherited fructose intolerance

For determining orocoecal transit time we recommend the lactulose-H₂-breath test, possibly supplemented by the lactose-[¹³C, ¹⁵N]ureide breath test for attaining a higher reliability of the diagnostic result. Lactose malabsorption can be best identified by the lactose-H₂-breath test. Intestinal malabsorption of carbohydrates in general is diagnosed using either the lactulose- or the xylose-H₂-breath test. For diagnosing sucrose intolerance we recommend the sucrose-H₂-breath test, while a positive glucose-H₂-breath test provides evidence of a bacterial overgrowth of the small intestine. Coeliac disease is diagnosed by means of the sorbitol-H₂-breath test.

6. GLOSSARY OF TERMS

Absorption:

Transfer of final food components into the intestinal mucosa

Bacterial overgrowth:

Too high a concentration of bacteria (in the small intestine)

Deficiency:

Lack of a biologically active substance

Digestion:

Decomposition of (commonly high molecular) food components by secretions of salivary glands, stomach, liver, pancreas and small bowel acting together

Disaccharidase deficiency:

Want of disaccharidases (in the small intestine)

Disaccharidases:

Enzymes, which cleave disaccharides to form corresponding monosaccharides. E. g. the disaccharide lactose in the small intestine is split up by lactase to form the monosaccharides galactose and glucose, which are absorbed in this part of the intestine.

Disaccharides:

Products of coupling two monosaccharides with one another in such a way that the aldehyde or keto group of one of the monosaccharides forms a glycoside with an OH-group of the other monosaccharide. Examples: Saccharose, composed of glucose and fructose; lactose, composed of galactose and glucose; maltose and trehalose, composed of two glucose molecules. Apart from the identity of the monosaccharide components disaccharides differ from each other in ring size of the respective semiacetals (5- or 6-membered rings) and in the manner these rings are connected with each other (α - or β -glycosidic connection).

Fructose:

Hexose with a keto group (keto-hexose) in position 2

Galactose:

Hexose with an aldehyde group (aldohexose)

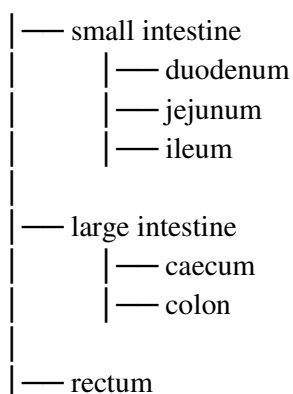
Glucose:

Hexose with an aldehyde group (aldohexose)

Hexoses:

Monosaccharides with six carbon atoms. Examples: glucose, fructose, mannose, galactose, sorbose

Intestine (gut, bowel):



Intolerance:

Incompatibility with lactose, fructose and some other substances used in connection with breath tests

Isomaltose:

Disaccharide from two glucose molecules formed by enzymatic degradation of starch or amylopectine

Lactose intolerance (hypolactasia):

Lactase deficiency (in the small intestine)

Lactulose:

Synthetic disaccharide of fructose and galactose, for which an enzyme splitting it into the

corresponding monosaccharides does not exist. Test substance for determining orocecal transit time by the corresponding H₂-breath test

Malabsorption:

Disorder of absorption of final food products through the intestinal wall

Mannose:

Hexose with an aldehyde group (aldohexose)

Monosaccharides:

(Mostly) linear chains from several, mostly five (pentoses) or six (hexoses) carbon atoms with one terminal aldehyde (—CHO-) group (aldoses) or a keto group in position 2 (ketoses), while the remaining carbon atoms except from hydrogen carry one —OH-group each

Oligosaccharides:

Products of coupling two (disaccharides) or several (trisaccharides, tetrasaccharides etc.) monosaccharides with one another in such a way that the aldehyde or keto group of a monosaccharide forms a glycoside with an —OH-group of a neighbouring monosaccharide

Optical activity:

All chemical compounds with one or more carbon atoms having four different neighbours (ligands) rotate the plane of polarised light either to the right or to the left, that is to say they are optically active, dextro-rotatory or lævo-rotatory. In view of the tetrahedral structure of such an asymmetric carbon atom the corresponding molecules exist in two different forms, the one being the mirror image of the other. These so-called optical isomers are found either in pure form, especially in their natural occurrences, or as an equimolecular mixture of both forms.

Optical isomers the molecular symmetry of which corresponds with the molecular symmetry of that isomer of tartaric acid, which rotates the plane of polarised light to the right, are named D-isomers, those corresponding with the other optical isomer of tartaric acid L-isomers. (Small letters d and l characterise the real sign of rotating the plane of polarised light, which is not necessarily identical with the sign of molecular symmetry itself. In case of tartaric acid this coincidence was brought about by definition.)

Pentoses:

Monosaccharides with five carbon atoms. Example: xylose

Polysaccharides:

Products of coupling many monosaccharides with one another in such a way, that the aldehyde or the keto group of one monosaccharide forms a glycoside with an —OH-group of a neighbouring monosaccharide. Examples: starch, cellulose

Sorbose:

Hexose with a keto group in position 2 (ketohehexose)

Sugars:

Crystalline mono- or oligosaccharides, readily soluble in water and tasting sweetly; in the narrower sense the disaccharide saccharose or sucrose obtained from sugar cane or sugar beets

Sweeteners:

1. Some water-soluble, sweetly tasting substances other than sugars, which are also fermented in the intestinal system forming H₂ (or CH₄) and therefore can be also used as test substances for H₂-(or CH₄-)breath tests (mannitol, sorbitol)

2. Water soluble, synthetic chemical compounds, the sweetening power of which considerably exceeds that of saccharose, glucose, maltose and other natural sugars. Not suitable for performing H₂-breath tests