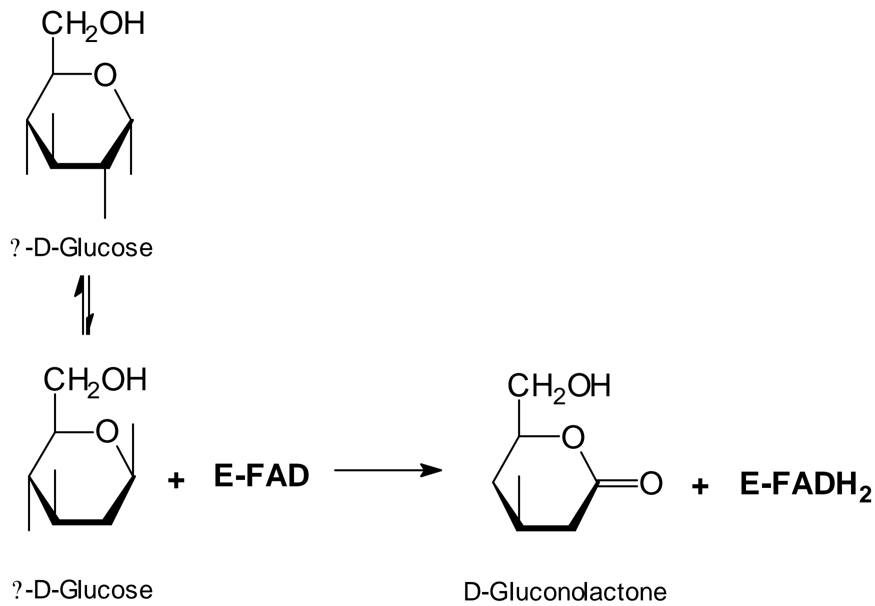
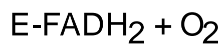
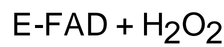
DETERMINATION OF BLOOD GLUCOSE LEVEL

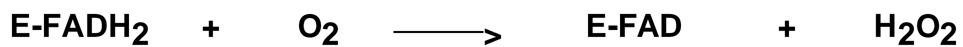
The level of blood glucose is kept in a very narrow range by hormonally and neurally controlled biochemical processes. This value in humans is 3.5-5.5 mM (80-100 mg%). If the blood glucose level is lower than the normal value we speak of hypoglycemia; when the level is higher than normal we call it hyperglycemia. In the past, blood glucose determinations were based on the redox property of glucose. The accuracy of these methods is not good because there are other components in the blood having redox properties. In addition *to* the other monosacharides (fructose or galactose), other compounds (uric acid or creatinine) can disturb the accurancy of the assay.

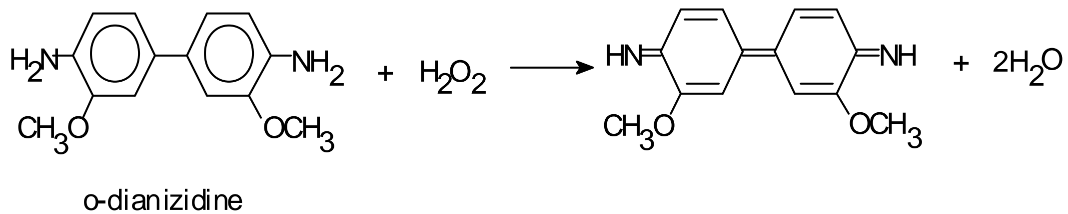
Recently, enzymatic methods, using the highly specific enzyme glucose oxidase, were developed for the routine determination of blood glucose level. The glucose oxidase (E.C. I.I.34) is a FAD coferment containing enzyme that catalyzes the oxidation of ?-D glucose to gluconolactone. It is isolated from molds, which also contain the mutarotase enzyme which enhances the conversion of ?-D glucose into the ß-D glucose form. As shown in the reaction scheme below, stoichiometric amount of H_2O_2 is also formed in the reaction. With the use of a third enzyme, peroxydase (E.C.I.II.I.7.), in a coupled reaction the H_2O_2 is transformed into H_2O while the necessary hydrogens are removed from an organic substrate molecule (e.g. ortho-dianisidine). The oxidized form of ortho-dianisidine is a coloured compound and its amount can be determined spectrophotometrically. The scheme of these reactions is summarized below:











o-dianizidine

Determination of the Blood glucose content of blood sample using glucose oxidase.

Solutions:

1.) glucose test reagents
2.) 0.5 mM glucose standard solution
3.) 0.33 M perchloric acid

Experiment:

The concentration of glucose can be estimated in blood samples taken from control and diabetic patients after 1g glucose/kg body weight oral glucose load at 0 min., 30 min., 60 min., 90 min. and 120 min.

Performance:

Pipet I ml perchloric acid into the eppendorf tube and add 0.1 ml blood sample to the tube. Mix it well and centrifuge it (5 min. at 5000 RPM) to remove the precipitated proteins. Aliquots of the supernatants are used in the assay . Samples are prepared according to the following table:

Blood glucose level after oral glucose load

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Supernatant; Omin;?l	50							
Supernatant; 3Omin;?l		50						
Supernatant; 6Omin;?l			50					
Supernatant; 9Omin;?l				5O				
Supernatant;12Omin;?l					50			
Glucose standard (0.5mM ?l), ?l						50	50	
H_2O ?l								50
Glucose reagent ml	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2

Incubation at 37 ?C, for 20 min.

Extinction 436 nm

Calculation:

$$\text{Blood glucose (mM)} = \frac{E_{sup}}{E_{gl\ st.}} * 0.5 * 11$$

Since the concentration of glucose standard = 0.5 mM and the dilution = 11 times.

The effect of insulin on the blood glucose level

Insulin, glucagon, epinephrine and norepinephrine are hormones regulating the storage and mobilisation of fuel molecules of metabolism. Insulin is produced by the ß-cells of the pancreas; its secretion is stimulated by glucose and by the parasympathetic nervous system. Insulin stimulates glycogen synthesis in both muscle and liver and supresses glyconeogenesis in the liver. The entry of glucose into muscle and adipose cells is also promoted by insulin. In addition, it activates the synthesis and storage of triacylglycerides in the adipose tissue. Insulin also has an effect on amino acid and protein metabolism.

Experiment:

Inject insulin (0.2 U/IO0 g of body weight) parenterally to fasted rats. Determine the blood glucose level before and 30 minutes after insulin administration using the glucose oxidase method described previously. (Note: draw the blood into a heparin containing test tube)