Sorbitol Dehydrogenase

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Sorbitol dehydrogenase (SDH), which was first described by *Blakley*[1], has been found in the liver and the genital organs of many mammals[2–4]. In man the liver has the largest concentration of SDH[5–7]; the concentration of the enzyme in other organs is given in Table 1. The serum and haemolysed blood of healthy human beings contains hardly any SDH[7]. The enzyme is also known as polyol dehydrogenase[7a].

Table 1. SDH activity in human tissues obtained at autopsy.

Tissue	SDH activity [units/mg. fresh weight]	SDH activity [units/mg. dry weight]
Liver	5.732	22.60
Prostate	1.366	8.46
Kidney	1.240	5.79
Spleen	0.453	2.07
Testes	0.190	1.28
Lymph nodes	0.212	0.93
Left ventricle	0.100	0.56
Skeletal muscle	0.113	0.49

Physiologically the cytoplasmic SDH may be considered to be coupled with a TPN-specific aldose reductase[8–9a] according to the following reactions:

(1) $$\text{D-Glucose} + \text{TPNH} + \text{H}^+ \overset{\text{reductase}}{\rightleftharpoons} \text{D-sorbitol} + \text{TPN}^+$$

(2) $$\text{D-Sorbitol} + \text{DPN}^+ \overset{\text{SDH}}{\rightleftharpoons} \text{D-fructose} + \text{DPNH} + \text{H}^+$$

The determination of SDH activity is of clinical importance[5–7, 10–13] because the sera of healthy individuals contain very little, but in liver cell damage (infectious, toxic or hypoxic in origin) it becomes demonstrable, therefore the SDH activity of serum can be of value as a fairly specific indicator of liver cell damage.

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Principle

The principle of the measurements is given in equation (2). The SDH activity can be measured in either direction but the use of fructose as substrate is more favourable for measurements in serum. The decrease in DPNH absorption with time at 340 or 366 mμ is a measure of the SDH activity.

Optimum Conditions for Measurements

Figure 1 shows the dependence of SDH activity in serum on the concentration of D-fructose. The optimum concentration is *ca.* 0.4 M (in triethanolamine buffer pH 7.4)[13,14].

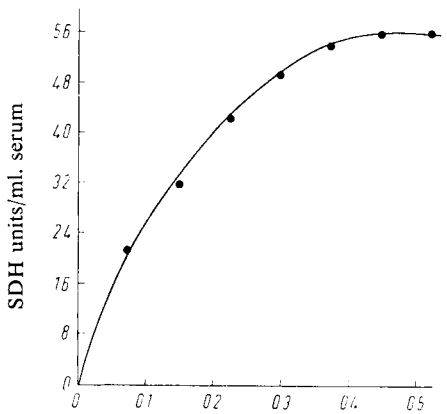

Fig. 1
Dependence of SDH activity in serum on the fructose concentration.

The pH optimum of the reaction with fructose in triethanolamine or tris-hydroxymethyl-aminomethane (tris) buffer is pH 6.1. The reaction is slightly faster in tris than in triethanolamine buffer, but in tris buffer a higher concentration of fructose is required to saturate the enzyme.

In several buffers the SDH reaction is 10—20 times faster with DPNH than with TPNH. Raising the temperature by 10° C, increases the reaction rate (between 20 and 35° C) by 150%[10].

The best working conditions for the measurement of SDH activity in human sera in different diseases are given in the following procedure.

Reagents *)

1. Triethanolamine hydrochloride
2. Reduced diphosphopyridine nucleotide, DPNH
   sodium salt, DPNH-Na_2; commercial preparation, see p. 1011.
3. D-Fructose, crystalline, pure
4. Sodium hydroxide, 2 N
5. Sodium hydrogen carbonate solution, 1% (w/v)

Preparations of Solutions

I. Triethanolamine buffer (0.2 M; pH 7.4):
Dissolve 3.72 g. triethanolamine hydrochloride in about 80 ml. distilled water, add 7.2 ml. 2 N NaOH, mix and dilute to 100 ml. with distilled water. Check pH with a glass electrode.

*) Complete reagent kits are available commercially, see p. 1037.

[14] *E. Schmidt* and *F. W. Schmidt*, personal communication.

II. Reduced diphosphopyridine nucleotide (1.2×10^{-2} M β-DPNH):
Dissolve 15 mg. DPNH-Na_2 in 1.5 ml. 1% $NaHCO_3$ solution.

III. D-Fructose (72% w/v):
Dissolve 72 g. fructose in distilled water and make up to 100 ml.

Stability of the solutions

The DPNH solution should be prepared freshly each week and stored at 2°–4° C. The other solutions are stable for a considerable period, as long as they are not contaminated with bacteria.

Procedure

Spectrophotometric measurements

Wavelength: 340 or 366 mμ; light path: 1 cm.; final volume: 3 ml.; temperature 24° C. Measure against air or water.

For each series of determinations a reagent blank is prepared containing water instead of serum.

Pipette successively into the cuvette:

1.6–2.4 ml. triethanolamine buffer (solution I)
0.1 ml. DPNH solution (II)
0.2–1.0 ml. serum.

Allow to stand *ca.* 30 min. until the optical density is constant (metabolites in the serum oxidize DPNH with the aid of dehydrogenases also present in the serum). Start the SDH reaction by addition of

0.3 ml. fructose solution (III).

Read the optical density at 30 or 60-second intervals for 5–8 min.

Calculations

A unit[3,7] is the amount of enzyme which causes an optical density change of 0.001/min. at 366 mμ and 24° C, in a final volume of reaction mixture of 3 ml. and with a 1 cm. light path. Measurements, made at 340 mμ are divided by 1.89 to allow for the difference in the extinction coefficient of DPNH at 366 and 340 mμ.

All optical density changes ΔE read at 30-second intervals are multiplied by 2 and these values or those read at 60-second intervals are averaged. From the definition of the activity unit it follows that:

$$\Delta E^{60\,\text{sec.}}_{366\,\text{m}\mu} \times 1000 = \text{SDH units/test mixture.}$$

The units per ml. of serum are obtained by dividing by the amount of serum used.

Under the test conditions the activity is proportional to the amount of serum added and the optical density changes are linear with time. The error with duplicate determinations, depending on the activity of the sample, is not more than 3%.

Multiplication of the SDH units/ml. serum by the factor 0.0545 gives the SDH activity in μmoles substrate reduced per ml. serum per hour at 24° C. The SDH activity in the serum of healthy people is < 1 unit/ml.

Stability of the Enzyme in the Serum Sample

The enzyme is relatively thermolabile. The decrease in activity[15] at different temperatures is given in Table 2.

Table 2. Stability of SDH in serum at different temperatures. The initial value is the activity of freshly collected serum[10].

Storage time	Loss of activity (as % of initial value) after storage of the serum sample at		
	+21°C	+4°C	−18°C
24 hr.	20.5	5.5	0
48 hr.	49	10.6	0

Sources of Error

SDH activity is inhibited by ethylene-diamine-tetra-acetate and mercury ions; mercaptans, sodium borate, mono-iodoacetate and KCN reduce the activity of the rat liver enzyme; heparin does not substantially affect the activity[15,19].

Details for Measurements in Tissues

For the determination of SDH activity in tissue[3,5–7a,9a,10,13,15–17] the sample is homogenized and centrifuged at high speed in the cold. The clear supernatant can be used directly for the assay, but the optimum conditions used for human serum measurements are not always valid for tissue preparations; other conditions apply in the case of purified SDH preparations[1,7a,9a,20,21]. With sorbitol as substrate, favourable conditions for measurements in rat liver extract are obtained by use of tris buffer (pH 9)[10,18] and 0.15 M sorbitol. Proportionality between activity and amounts of enzyme added, as well as a linear increase in optical density with time are obtained. In the opposite direction the reaction (with 0.4 M fructose) proceeds about 8 times faster in triethanolamine buffer (pH 7.4) and about 5 times faster in tris buffer (pH 7.4).

It is to be noted that the estimation of liver SDH usually includes cytoplasmic SDH and the mitochondrial xylitol dehydrogenase. Both enzymes are practically homospecific, but are not identical[9a].

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