Glutamate-Oxaloacetate Transaminase

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Glutamate-oxaloacetate transaminase (GOT) has been detected in micro-organisms and in all human and animal tissues so far investigated. In humans[1] the richest source is heart muscle, followed by*): brain, liver, gastric mucosa, adipose tissue, skeletal muscle, kidney, *etc.*, and finally serum with substantially smaller amounts.

Several possibilities exist for the measurement of activity[2]: paper chromatography of the substrates or reaction products after incubation (refer to equation 1); spectrophotometric measurement of the oxaloacetate formation at 280 mμ; manometric determination of, for example, glutamic acid with glutamic acid decarboxylase. The most widely used methods involve the measurement of the oxaloacetate formed from aspartate and α-oxoglutarate: 1. by an enzymatic indicator reaction with malic dehydrogenase and reduced diphosphopyridine nucleotide (DPNH); 2. by the use of 2,4-dinitrophenylhydrazine as a ketone trapping reagent.

The pyridoxal phosphate required as coenzyme[3,4] is present in sufficient quantities in serum and all tissue samples.

Determination with Malic Dehydrogenase as Indicator Enzyme

Principle

Glutamate-oxaloacetate transaminase (GOT) catalyses the reaction:

(1) L-Glutamate + oxaloacetate $\rightleftharpoons$ L-aspartate + α-oxoglutarate

The activity of the transaminase is measured by the increase of oxaloacetate with time as the reaction proceeds from right to left. The oxaloacetate is determined with the indicator reaction catalysed by malic dehydrogenase (MDH):

(2) Oxaloacetate + DPNH + H^+ $\rightleftharpoons$ malate + DPN^+

The oxidation of DPNH, which is proportional to the amount of oxaloacetate formed, is measured by the decrease in the optical density at 340 or 366 mμ. The following description for the measurement of transaminase activity in serum is essentially that of *Karmen*[5], although the substrate concentrations have been modified[6].

Optimum Conditions for Measurements

The optimum pH and substrate concentrations have been investigated by several workers[5,7]. In human serum the following relationship has been established for the enzyme originating from heart (after myocardial infarction) and from liver (in hepatitis)[6]: In the pH range 6—8.5 the activity is dependent on the aspartate concentration, but practically independent of the α-oxoglutarate concentration. With aspartate concentrations $> 2 \times 10^{-1}$ M there is a wide activity optimum between pH 7 and 8.5. The activities measured with various substrate concentrations at pH 7.6 and 25°C are given in

*) Units/mg. tissue protein; if related to the fresh weight of tissue the order is different.

1) *F. W. Schmidt*, Lecture Marburg/Lahn, Germany, Dec. 1959.
2) See *P. P. Cohen* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1955, Vol. II, p. 178.
3) *D. E. Green, L. F. Leloir* and *V. Nocito*, J. biol. Chemistry *161*, 559 [1945].
4) *D. O'Kane* and *I. C. Gunsalus*, J. biol. Chemistry *170*, 425 [1947].
5) *A. Karmen*, J. clin. Invest. *34*, 131 [1955].
6) *H.-U. Bergmeyer* and *E. Bernt*, unpublished.
7) *E. Schmidt* and *F. W. Schmidt*, personal communication.

Figure 1. Optimum values are obtained in phosphate buffer with α-oxoglutarate concentrations from 6×10^{-3} to 10^{-2} M and aspartate concentrations from 2×10^{-1} to 5×10^{-1} M. Variation of the aspartate concentration from 3×10^{-2} to 3×10^{-1} M in relation to the α-oxoglutarate concentration only leads to a slight alteration of the optimum activity and *vice versa*.

Approximately optimum and technically favourable conditions for assay in normal serum after myocardial infarction and in hepatitis are: phosphate buffer pH 7.6, *ca.* 7×10^{-3} M α-oxoglutarate and 2×10^{-1} M aspartate. A sufficiently rapid indicator reaction is assured by using 2×10^{-4} M DPNH and 25 μg. malic dehydrogenase per 3 ml. assay mixture.

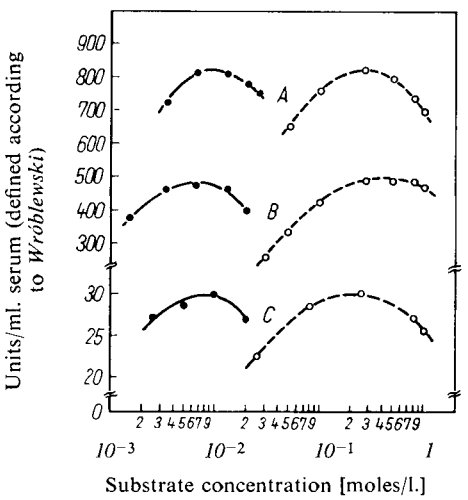

Fig. 1. Dependence of the GOT activity on the substrate concentration.
Solid line: Variation of α-oxoglutarate with a constant amount of aspartate (2×10^{-1} M).
Broken line: Variation of aspartate with a constant amount of α-oxoglutarate (6.7×10^{-3} M).
pH 7.6; 25°C.
Curves A: Serum after hepatitis, B: Serum after myocardial infarction, C: Normal serum. Each pair of curves was measured with the same serum.

Reagents *)

1. Potassium dihydrogen phosphate, A. R., KH_2PO_4
2. Dipotassium hydrogen phosphate, A. R., K_2HPO_4
3. L-Aspartic acid
   sodium salt or free acid
4. α-Oxoglutaric acid
   sodium salt or free acid; commercial preparation, see p. 1024.
5. Reduced diphosphopyridine nucleotide, DPNH
   sodium salt, DPNH-Na_2. Commercial preparation, see p. 1011.
6. Malic dehydrogenase, MDH
   from pig heart, suspension in 2.8 M ammonium sulphate solution. Commercial preparation, see p. 988.

Purity of the indicator enzyme

The MDH preparation should have a specific activity of at least 2000 units/mg. according to *Bücher*[8], corresponding to > 7500 units/mg. according to *Ochoa*[9] or > 35 units/mg. according

*) Complete reagent kits are available commercially, see p. 1036.

[8] *G. Beisenherz, J. H. Boltze, Th. Bücher, R. Czok, K. H. Garbade, E. Meyer-Arendt* and *G. Pfleiderer*, Z. Naturforsch. *8b*, 555 [1953].

[9] *S. Ochoa* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1955, Vol. I, p. 735.

to *Racker*[10]. It should not be contaminated by more than: 0.03% glutamate-oxaloacetate transaminase (measured with *ca.* 0.2 μg. pyridoxal phosphate per assay) or 0.03% glutamic dehydrogenase (relative to the specific MDH activity).

Preparation of Solutions (for *ca.* 20 determinations)

To prevent the growth of micro-organisms, sterilize the containers.

I. Phosphate-aspartate solution (0.1 M phosphate buffer pH 7.6; 2.5×10^{-1} M aspartate):

Dissolve 0.175 g. KH_2PO_4, 1.742 g. K_2HPO_4 and *ca.* 3.9 g. Na-L-aspartate or 3.3 g. L-aspartic acid in doubly distilled water and make up to 100 ml. If free aspartic acid is used adjust the pH of the solution to 7.6 with 1 N NaOH before dilution.

II. α-Oxoglutarate (*ca.* 0.2 M):

Dissolve *ca.* 114 mg. Na-α-oxoglutarate or 88 mg. α-oxoglutaric acid in doubly distilled water and make up to 3 ml. If the free acid is used adjust to pH 7.6 with 1 N NaOH before dilution.

III. Reduced diphosphopyridine nucleotide (1.2×10^{-2} M β-DPNH):

Dissolve 15 mg. DPNH-Na_2 in 1.5 ml. solution I.

IV. Malic dehydrogenase, MDH (0.5 mg. protein/ml.):

Dialyse the enzyme suspension in ammonium sulphate solution for three periods of 4 hours at 0°C against 200 volumes of 0.01 M potassium phosphate (pH 7.6) each time. Dilute the contents of the dialysis sac with glycerol to give 50% (v/v) glycerol-water and 0.5 mg. protein/ml.

Stability of the solutions

Store all solutions, stoppered, in a refrigerator at 0 to 4°C. Prepare the DPNH solution freshly each week and the α-oxoglutarate solution each fortnight. The remaining solutions are stable almost indefinitely. Deterioration of the solutions, especially the phosphate-aspartate solution, is usually caused by bacterial contamination. This can be prevented by the addition of 0.3 ml. chloroform. Pour out the day's requirement of the solution; do not use a pipette.

Procedure

Spectrophotometric measurements

Use only fresh serum free from haemolysis. If the serum to be examined is strongly coloured prepare a blank containing 2.5 ml. water + 0.5 ml. serum or 3 ml. water + 0.03 ml. DPNH solution (III). Measure against this blank. Otherwise measure against air or doubly distilled water.

Wavelength: 340 mμ or 366 mμ; light path: 1 cm.; final volume: 3.0 ml.; temperature: 25°C (constant temperature cuvette holder).

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[10] *J. Cooper, P. A. Srere, M. Tabachnick* and *E. Racker*, Arch. Biochem. Biophysics *74*, 306 [1958].

Pipette successively into the cuvette:

2.30 ml. phosphate-aspartate solution (I)
0.05 ml. DPNH solution (III)
0.05 ml. MDH solution (IV)
0.50 ml. serum.

Mix with a glass or plastic rod flattened at one end and equilibrate for *ca.* 5–10 min. *). Mix in

0.10 ml. α-oxoglutarate solution (II)

to start the reaction. After *ca.* 1 min. start a stopwatch and read the optical density every 2 min. for a period of 10 min. With very high GOT activity a period of 2 to 4 min. is sufficient. ΔE/min. (366 mμ) should not exceed 0.030; if necessary, dilute the serum 5 to 10 times with solution I.

The traces of GOT in the MDH preparation should be determined for each MDH preparation. For this, doubly distilled water containing 0.1 μg. pyridoxal phosphate is substituted for serum and measurements are made against a cuvette containing doubly distilled water (blank).

Modification of the procedure

1. If a constant temperature cuvette holder is not available, mix solutions I, III, IV and the serum in a test tube and equilibrate in a water bath at 25°C for 10 min. Start the transaminase reaction by mixing in the α-oxoglutarate solution (II) and pour the mixture into a cuvette for the measurements. It is assumed that the room temperature is similar to the temperature of the measurements and that during the measurements the reaction mixture will not cool by more than 1–2°C (error <10%).
2. To save time in a series of measurements proceed as described under 1. but measure the initial optical density immediately after the addition of α-oxoglutarate, pour the reaction solution back into the test tube, after 10 min. pour the reaction solution once more into the cuvette and read the final optical density. This procedure is valid, because a) volume losses need not be considered, b) the time course of the GOT reaction is linear and c) the temperature remains more constant than in the first case.

Calculations

Calculate the mean of the measured ΔE/min. values and subtract from this the blank value due to any traces of transaminase in the MDH preparation. Use the difference for the calculations.

According to[11] a unit is the amount of transaminase in 1 ml. serum, which decreases the optical density of DPNH at 340 mμ by 0.001 in 1 min., in a 3 ml. assay mixture at 25°C. It follows that with 0.5 ml. serum:

$$(\Delta E_{340}/\text{min.}) \times 1000 \times 2 = \text{GOT units/ml. serum.}$$

For measurements at 366 mμ it is necessary to multiply by 1.89 because of the ratio of the extinction coefficients of DPNH at 340 and 366 mμ. Therefore:

$$(\Delta_{340}/\text{min.}) \times 2000 = (\Delta E_{366}/\text{min.}) \times 3780 = \text{GOT units/ml. serum.}$$

*) Pyruvic acid, oxaloacetic acid and other substrates react with the respective DPN-linked enzymes in the serum resulting in the oxidation of DPNH. After preliminary incubation and addition of α-oxoglutarate only the transaminase reaction occurs.

[11] *J. S. LaDue, F. Wróblewski* and *A. Karmen*, Science [Washington] *120*, 497 [1945].

Example

When 0.5 ml. normal serum was assayed the following optical densities were measured at 366 mμ:

before addition of α-oxoglutarate	0.310	
after addition of α-oxoglutarate	0 min. 0.315	
	2 min. 0.303	$\Delta E = 0.012$
	4 min. 0.288	$\Delta E = 0.015$
	6 min. 0.278	$\Delta E = 0.010$
	8 min. 0.265	$\Delta E = 0.013$
	10 min. 0.252	$\Delta E = 0.013$
	Mean:	$\Delta E = 0.0126/2$ min.
		$\Delta E = 0.0063$/min.

Blank of the MDH preparation: ΔE_{Blk}/min. = 0.001/min.; ΔE/min. − E_{Blk}/min. = 0.0053/min.

$0.0053 \times 3780 = 20$ GOT units/ml. serum.

Conversion to other units

According to *Bücher et al.*[8] a unit of DPN or TPN-dependent dehydrogenases is the amount of enzyme contained in 1 ml., which alters the optical density of DPNH (TPNH) at 366 mμ by 0.100 in 100 sec., with a light path of 1 cm. and at 25°C. Therefore:

1 unit (*Bücher*): ΔE_{366}/100 sec. = 0.100/100 sec. 1 unit (*Wróblewski*): ΔE_{340}/min. = 0.001/min.

ΔE_{366}/min. = 0.060/min.

ΔE_{340}/min. = 0.113/min.

for an assay volume of 3 ml.: ΔE_{340}/min. = 0.0377/min.

37.7 units (*Wróblewski*) = 1 unit (*Bücher*); 0.0265 units (*Bücher*) = 1 unit (*Wróblewski*).

To calculate directly from the measured optical density difference:

for 340 mμ: $(\Delta E/\text{min.}) \times 2000 =$ units (*Wróblewski*)

$(\Delta E/\text{min.}) \times 2000 \times 0.0265 = (\Delta E/\text{min.}) \times 53 =$ units (*Bücher*)

for 366 mμ: $(\Delta E/\text{min.}) \times 3780 =$ units (*Wróblewski*)

$(\Delta E/\text{min.}) \times 3780 \times 0.0265 = (\Delta E/\text{min.}) \times 101 =$ units (*Bücher*)

For conversion to other units, see p. 33.

Stability of the Enzyme in the Serum Sample

According to[12] the GOT activity in serum decreases by about 22% after 24 hours at room temperature, by about 15% at +4°C and by about 5% at −20°C.

Sources of Error

The presence of glutamic dehydrogenase in the sample interferes with the reaction, because it catalyses the reduction of α-oxoglutarate with the simultaneous oxidation of DPNH. Glutamic dehydrogenase is barely present in normal serum, but in pathological serum it may be present in significant amounts. If the presence of ammonium ions is avoided in the assay (by use of a solution of MDH in glycerol-water), then the glutamic dehydrogenase reaction cannot proceed. The Michaelis constant of this enzyme for ammonium ions is so large (5.6×10^{-2} M) that it remains inactive even with the raised ammonia concentrations which occur in some pathological sera.

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[12] *H. Südhof* and *E. Wötzel*, Klin. Wschr. *38*, 1165 [1960].

Effects of Therapeutic Agents

Therapy with cortisone and its derivatives significantly decreases the raised serum GOT activity in hepatitis within a few hours.

Details for Measurements in Tissue

According to *Bücher et al.*[13] GOT belongs to both the intra- and extramitochondrial located enzymes. Simple homogenization in a *Potter-Elvehjem* apparatus (refer to p. 49) is not sufficient to completely extract the enzyme. The activity is therefore determined in a homogenate prepared according to[14], but the measured units are related to the soluble protein in the supernatant as determined by the biuret method after centrifuging at high speed. For measurements on, for example, liver punctures, 1 mg. fresh weight is sufficient.

The optimum conditions for human serum are not necessarily valid for sera from other species or for other tissues. The position of the activity optima for human serum shown in Fig. 1 may be displaced considerably. In preliminary experiments on mouse liver homogenate[5] two to five times higher concentrations of L-aspartate and α-oxoglutarate were necessary to obtain optimum activity.

Animal organs, especially liver, kidney and brain[1] are rich in glutamic dehydrogenase. It is necessary to determine the amount of interference due to this enzyme, since this occurs in spite of the use of ammonia-free reagents: the phosphate-aspartate buffer is substituted by one containing α-oxoglutarate and the GOT reaction is started with L-aspartate after measurements of the glutamic dehydrogenase activity. The difference of the two ΔE/min. values is used for the calculation of the GOT activity.

Colorimetric Determination with 2,4-Dinitrophenylhydrazine

The GOT activity can be measured colorimetrically by the use of two points, because the course of the reaction is linear with time. The first colorimetric method was described by[15]: The reaction product oxaloacetate was quantitatively decarboxylated to pyruvate with aniline citrate as catalyst and the pyruvate was determined as the 2,4-dinitrophenylhydrazone. Some later workers (*e.g.*[16]) rejected this method in favour of a more simplified procedure, although other workers (*e.g.*[17–19]) retained it. The simplified procedure has proved more successful (*e.g.*[20]).

Principle

After a fixed time the 2,4-dinitrophenylhydrazone of the reaction product, oxaloacetate, formed according to equation (1), (see p. 837), is determined spectrophotometrically in alkaline solution. Some of the oxaloacetate decarboxylates spontaneously to pyruvate. The assay mixture therefore contains oxaloacetate, pyruvate and α-oxoglutarate, all of which form 2,4-dinitrophenylhydrazones with absorption maxima at different wavelengths (Fig. 2). In order to keep the contribution of the α-oxoglutarate hydrazone to the colour low, the measurements are made at wavelengths (about 500 to 550 mμ) higher than the wavelength of maximum absorption since this allows the greatest differentiation between the optical densities of the three hydrazones.

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[13] *Th. Bücher* and *P. Baum*, Lecture Berlin 1958.
[14] *E. Schmidt, F. W. Schmidt* and *E. Wildhirt*, Klin. Wschr. *36*, 179 [1958].
[15] *N. E. Tonhazy, N. G. White* and *W. W. Umbreit*, Arch. Biochem. Biophysics *28*, 36 [1950].
[16] *S. Reitman* and *S. Frankel*, Amer. J. clin. Pathol. *28*, 56 [1956].
[17] *P. Cabaud, R. Leeper* and *F. Wróblewski*, Amer. J. clin. Pathol. *26*, 1101 [1956].
[18] *U. C. Dubach*, Schweiz. med. Wschr. *87*, 185 [1957].
[19] *D. F. Ashman*, Acta ci. venezolana *10*, 14 [1959].
[20] *A. P. Hansen*, Nordisk Med. *61*, 799 [1959].

The extinction coefficients of the 2,4-dinitrophenylhydrazones of pyruvate or oxaloacetate cannot be used for the calculation of the experimental results for the reason which has been explained above. The method is best standardized by direct comparison with the spectrophotometric method using MDH as indicator enzyme (see above). For routine use it is sufficient to use standards containing

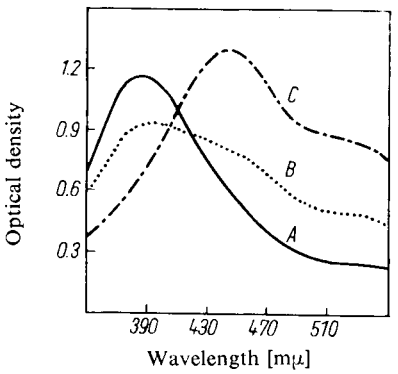

Fig. 2. Absorption spectra of equimolar alkaline solutions of the 2,4-dinitrophenylhydrazones of (A) α-oxoglutarate, (B) oxaloacetate and (C) pyruvate (according to [16]).

different amounts of pyruvate, but with a constant molecular amount of pyruvate + α-oxoglutarate. The standard curves (abscissa: GOT activity; ordinate: optical density) are non-linear. Oxaloacetate is not suitable as a standard because of its instability in aqueous solution.

Reagents *)

1. Potassium dihydrogen phosphate, A. R., KH_2PO_4
2. Dipotassium hydrogen phosphate, A. R., K_2HPO_4
3. L-Aspartic acid
   sodium salt or free acid.
4. α-Oxoglutaric acid
   sodium salt or free acid; commercial preparation, see p. 1024.
5. Sodium pyruvate
   commercial preparation, see p. 1027.
6. 2,4-Dinitrophenylhydrazine
7. Hydrochloric acid, A. R., 1 N
8. Sodium hydroxide, A. R., as pellets

Preparation of Solutions (for *ca.* 50 determinations)

To prevent the growth of micro-organisms, sterilize the containers.

I. Substrate-buffer solution (0.1 M phosphate buffer, pH 7.4; 0.1 M L-aspartate; 2×10^{-3} M α-oxoglutarate):
   Dissolve 1.50 g. K_2HPO_4, 0.20 g. KH_2PO_4, 0.039 g. Na-α-oxoglutarate or 0.030 g. α-oxoglutaric acid and 1.57 g. Na-L-aspartate or 1.32 g. L-aspartic acid in <100 ml. doubly distilled water. Adjust the pH to 7.4 with 0.4 N NaOH (solution III) using a glass electrode and dilute to 100 ml. with doubly distilled water.

II. Ketone reagent (10^{-3} M 2,4-dinitrophenylhydrazine):
   Dissolve 20 mg. 2,4-dinitrophenylhydrazine in 1 N HCl and make up to 100 ml.

*) Complete reagent kits are commercially available, see p. 1036.

III. Sodium hydroxide (0.4 N):
Dissolve 16 g. NaOH in doubly distilled water and make up to 1000 ml.

IV. Sodium pyruvate (2×10^{-3} M):
Dissolve 22 mg. Na pyruvate in doubly distilled water and make up to 100 ml.

Stability of the solutions

The NaOH and ketone reagent are stable indefinitely if well stoppered. Store the substrate-buffer and pyruvate solution at 0—4° C. So long as the growth of micro-organisms is prevented (by addition of a few drops of chloroform) the solutions are stable for at least a month.

Procedure

Use only fresh serum free from haemolysis.

Enzymatic reaction

Wavelength: 530 or 546 mμ; light path: 1 cm.; temperature: 37°C (water bath). A blank in which the serum is added after the incubation is required for each sample. Measure against this blank.

Pipette into test tubes:

Experimental	*Blank*
1 ml. substrate-buffer solution (I)	1 ml. substrate-buffer solution (I)
0.2 ml. serum	—
mix by inversion and incubate for exactly 60 min. Add	do not incubate. Add
1 ml. ketone reagent (soln. II)	1 ml. ketone reagent (soln. II)
—	0.2 ml. serum

Allow to stand for 20 min. at room temperature. Mix into the experimental and blank tubes

10 ml. NaOH (solution III)

and after 5 min. read the optical density of the experimental tube against the blank. With results of over 150 units repeat the measurements with serum diluted 1 :10 with physiological saline.

Standard curve and table of values

Pipette successively into test tubes:

Test tube No.	Na pyruvate solution (IV) [ml.]	Buffer-substrate solution (I) [ml.]
1	0.0	1.0
2	0.05	0.95
3	0.10	0.90
4	0.15	0.85
5	0.20	0.80
6	0.25	0.75

Pipette into each test tube:

0.2 ml. water

1.0 ml. ketone reagent (solution II).

Mix and allow to stand for 20 min. at room temperature. Add

10.0 ml. NaOH (solution III)

to each tube, mix and after 5 min. read the optical densities against tube No. 1. Plot the optical densities (ordinate) against GOT units (abscissa). By comparison with the spectrophotometric method (MDH as indicator enzyme) the following relationship was found:

Test tube No. 2 = 21 GOT units/ml. serum
Test tube No. 3 = 42 GOT units/ml. serum
Test tube No. 4 = 64 GOT units/ml. serum
Test tube No. 5 = 97 GOT units/ml. serum
Test tube No. 6 = 140 GOT units/ml. serum

By direct comparison of the two methods the following table was constructed for measurements at 546 mμ.

Optical density	Units (according to[10])	Optical density	Units (according to[10])
0.020	8	0.160	71
0.040	16	0.180	83
0.060	24	0.200	100
0.080	32	0.220	118
0.100	40	0.240	140
0.120	49	0.260	167
0.140	59		

Calculations

Either read off the units for the measured optical densities from the standard curve, or for measurements at 546 mμ obtain the units from the above table.