Glutamate-Pyruvate Transaminase

Hans-Ulrich Bergmeyer and Erich Bernt

The glutamate-pyruvate transaminase (GPT) content of human tissue (activity relative to fresh weight) decreases in the following order[1,2]: liver, kidney, heart, skeletal muscle, pancreas, spleen, lung, serum.

The activity can be determined by several methods: by paper chromatography of the substrates or reaction products, by manometric determination of the glutamic acid formed with glutamic acid decarboxylase, or by colorimetric determination of the pyruvate formed with salicylaldehyde. The most successful methods depend on the measurement of the pyruvate formed from alanine and α-oxoglutarate: 1. enzymatically with lactic dehydrogenase and reduced diphosphopyridine nucleotide (DPNH); 2. colorimetrically with 2,4-dinitrophenylhydrazine. According to[5] the diphosphopyridine nucleotide (DPN) formed in the indicator reaction (equation 2) can be determined fluorimetrically.

The coenzyme of GPT is pyridoxal phosphate[3]; this is firmly bound to the enzyme protein (in contrast to glutamate-oxaloacetate transaminase)[4], so that pyridoxal phosphate need not be added to the assay mixture.

Determination with Lactic Dehydrogenase as Indicator Enzyme

This method was first described by *Henley et al.*[6]. The following description is based on the method of *Wróblewski* and *La Due*[2].

Principle

Glutamate-pyruvate transaminase (GPT) catalyses the reaction:

(1) L-Glutamate + pyruvate $\rightleftharpoons$ L-alanine + α-oxoglutarate

The activity of the transaminase is measured by the increase of pyruvate with time. The pyruvate is determined by the indicator reaction catalysed by lactic dehydrogenase (LDH):

(2) Pyruvate + DPNH + H^+ $\rightleftharpoons$ lactate + DPN^+

The rate of oxidation of reduced diphosphopyridine nucleotide (DPNH) is proportional to the increase of pyruvate with time. The decrease of optical density at 340 or 366 mμ due to the oxidation of DPNH is measured.

Optimum Conditions for Measurements

The optimum pH and optimum substrate concentration for the GPT reaction have been measured for serum[5,7], but no systematic study has been made of the interdependence of these factors and whether they are the same for normal and pathological sera. It has been discovered[8] that none of the present methods are carried out under optimum conditions. All the GPT values given in the literature are therefore more than 50% too low. This is important when comparing the activity with that of other enzymes, for example, glutamate-oxaloacetate transaminase (GOT) ("De Ritis quotient", p. 666, 671, consideration of the enzyme make-up of cells: p. 662), but for clinical work it is less important. For this reason both the conventional and optimum methods are described below.

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1) *F. Wróblewski* and *J. S. LaDue*, Ann. intern. Med. *45*, 801 [1956].
2) *F. Wróblewski* and *J. S. LaDue*, Proc. Soc. exp. Biol. Med. *91*, 569 [1956].
3) *D. E. Green*, *L. F. Leloir* and *V. Nocito*, J. biol. Chemistry *161*, 559 [1945].
4) *L. Grein* and *P. Pfleiderer*, Biochem. Z. *330*, 433 [1958].
5) *T. Laursen* and *P. F. Hansen*, Scand. J. Clin. Lab. Invest. *10*, 53 [1958].
6) *K. S. Henley* and *H. M. Pollard*, J. Lab. clin. Med. *46*, 785 [1955].
7) *E. Schmidt* and *F. W. Schmidt*, personal communication.
8) *E. Bernt* and *H.-U. Bergmeyer*, unpublished.

The pH optimum at 25° C was found to be from 7.3 to 7.8 according to the substrate concentration. With normal serum in *ca.* 0.06 M phosphate buffer and with L-alanine concentrations from 3×10^{-2} to 1 M the optimum for activity lay between 3×10^{-3} and 3×10^{-1} M α-oxoglutarate. The differences are not large and occasionally cannot be detected. In hepatitis serum significant differences are always observed; optimum activity was found with 2×10^{-2} to 5×10^{-2} M α-oxoglutarate. Variation of the L-alanine concentration with constant α-oxoglutarate (3×10^{-2} M) indicated an optimum concentration of about 8×10^{-1} M L-alanine for normal serum and about 1.2 M L-alanine for hepatitis serum.
Approximately optimum conditions for measurements in normal serum and serum after hepatitis are phosphate buffer (0.06 M; pH 7.4), *ca.* 3×10^{-2} M α-oxoglutarate and *ca.* 1 M L-alanine. A sufficiently rapid indicator reaction is guaranteed with 2×10^{-4} M DPNH and 25 μg. lactic dehydrogenase per 3 ml. assay mixture.
With the conventional method the assay mixture contains about 6.6×10^{-3} M α-oxoglutarate and 3.3×10^{-2} M L-alanine (the use of DL-alanine is permissible, since D-alanine does not inhibit the reaction).

Conventional Method

The procedure described here for the measurements in serum is that of *Wróblewski* and *La Due*[2]

Reagents*)

1. Potassium dihydrogen phosphate, KH_2PO_4, A. R.
2. Dipotassium hydrogen phosphate, K_2HPO_4, A. R.
3. DL-Alanine
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. Lactic dehydrogenase, LDH
   crystalline, from skeletal muscle; suspension in 2.2 M ammonium sulphate solution. Commercial preparation, see p. 986.

   Purity of the indicator enzyme (LDH)
   The specific activity of the LDH preparation should be at least 15000 units/mg. according to *Bücher et al.*[9] or 270 units/mg. according to *Racker et al.*[10]. It should not be contaminated by more than 0.03% glutamate-pyruvate transaminase or 0.03% glutamic dehydrogenase (relative to the specific activity of the LDH).

Preparation of Solutions (for *ca.* 25 determinations)

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

I. Phosphate-alanine solution (0.1 M phosphate buffer, pH 7.4; 0.11 M DL-alanine):
   Dissolve 0.2 g. KH_2PO_4, 1.5 g. K_2HPO_4 and 1.0 g. DL-alanine in doubly distilled water and make up to 100 ml.

II. α-Oxoglutarate (*ca.* 0.2 M):
   Dissolve 114 mg. Na-α-oxoglutarate or 87.5 mg. α-oxoglutaric acid in *ca.* 2 ml. doubly distilled water, adjust to pH 7.4 with 1 N NaOH and dilute to 3 ml. with doubly distilled water.

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

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

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

III. Reduced diphosphopyridine nucleotide (*ca.* 1.2×10^{-2} M β-DPNH):
Dissolve 15 mg. DPNH-Na_2 in 1.5 ml. solution I.

IV. Lactic dehydrogenase, LDH (0.5 mg. protein/ml.):
Dialyse the enzyme suspension in ammonium sulphate solution against 0.01 M potassium phosphate buffer (pH 7.6). Dilute the contents of the dialysis sac with glycerol to give 50% (v/v) glycerol 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 every fortnight. The other solutions are stable practically indefinitely. Bacterial contamination causes deterioration of the solutions, especially the phosphate-alanine solution, but this can be prevented by the addition of 0.3 ml. chloroform. The daily requirement of reagents should be obtained by pouring, and not with a pipette.

Procedure

Spectrophotometric measurements

Use only fresh serum free from haemolysis. If the serum to be assayed is strongly coloured prepare a control cuvette containing 2 ml. doubly distilled water and 1 ml. serum or 3 ml. doubly distilled water and 0.03 ml. DPNH solution (III). Measure against this control cuvette. Otherwise measure against air or doubly distilled water.

Wavelength: 340 or 366 mμ; light path: 1 cm.; final volume: 3 ml.; temperature: 23° C (constant temperature cuvette holder).

Pipette successively into the cuvette:

1.82 ml. phosphate-alanine solution (I)
0.04 ml. DPNH solution (III)
0.04 ml. LDH solution (IV)
1.00 ml. serum.

Mix with a glass rod flattened at one end and equilibrate for 5 – 10 min. During this period the pyruvate contained in the serum reacts with DPNH and LDH. Mix in

0.10 ml. α-oxoglutarate solution (II)

to start the transaminase reaction. After *ca.* 1 min. start a stopwatch and read the optical density at 2 min. intervals for 10 min. With high GPT activity a period of 2 – 4 min. is sufficient. The ΔE/min. at 366 mμ should not be greater than 0.030; if necessary, dilute the serum 5 to 10 times with solution I. The traces of GPT in the LDH preparation should be determined for each LDH preparation. For this, replace the serum by doubly distilled water.

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 for *ca.* 10 min. in a water bath at 23° C. Start the transaminase reaction by mixing in the α-oxoglutarate solution (II) and then pour the reaction 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 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 GPT reaction is linear and c) the temperature remains more nearly constant than in the first case.

Calculations

According to the definition of *Wróblewski* and *La Due* [2] 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 23° C. It follows that with 1.0 ml. serum:

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

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:

$$(\Delta E_{340}/\text{min.}) \times 1000 = (\Delta E_{366}/\text{min.}) \times 1890 = \text{GPT units/ml. serum.}$$

Calculate the mean of the measured ΔE/min. values and subtract from this the blank value due to any traces of transaminase in the LDH preparation. Use the difference for the calculations. For normal values, see p. 704.

Example

When 1 ml. normal serum was assayed the following optical densities were measured at 366 mμ:

Before addition of α-oxoglutarate		0.413	
After addition of α-oxoglutarate	0 min.	0.405	
			ΔE = 0.012/2 min.
	2 min.	0.393	
			ΔE = 0.015/2 min.
	4 min.	0.378	
			ΔE = 0.014/2 min.
	6 min.	0.364	
			ΔE = 0.014/2 min.
	8 min.	0.350	
			ΔE = 0.014/2 min.
	10 min.	0.336	
		Mean	ΔE = 0.014/2 min.
			ΔE/min. = 0.007

Blank of the LDH preparation: ΔE/min. = 0.0005

$(0.007 - 0.0005) \times 1890 = 0.0065 \times 1890 = 12.3$ GPT units/ml. serum.

Conversion to other units

1. For dehydrogenases and enzymes which react with dehydrogenases in coupled reactions, a unit according to *Bücher et al.* [9] is the amount of enzyme contained in 1 ml. which changes the optical density of DPNH at 366 mμ by 0.100 in 100 sec. at 25° C, with a light path of 1 cm.

Therefore

1 unit (*Bücher*): $\Delta E_{366}/100$ sec. = 0.100; 1 unit (*Wróblewski*): $\Delta E_{340}/\text{min.} = 0.001$

$$\Delta E_{366}/\text{min.} = 0.060$$
$$\Delta E_{340}/\text{min.} = 0.113$$

for a 3 ml. assay mixture: $\Delta E_{340}/\text{min.} = 0.0377$

37.7 units (*Wróblewski*) = 1 unit (*Bücher*)

0.0265 units (*Bücher*) = 1 unit (*Wróblewski*)

2. According to *Racker et al.* [10] a unit is the amount of enzyme which converts 1 μmole of substrate in 1 min. at 25° C.

Therefore

1 unit (*Racker*): 1 μmole/min.; 1 unit (*Wróblewski*): $\Delta E_{340}/\text{min.} = 0.001$.

Since ΔE_{340}/min. = 0.001 corresponds to a conversion of 4.82×10^{-4} μmoles substrate/3 ml. (3 ml. assay mixture),

1 unit (*Wróblewski*) = 4.82×10^{-4} units (*Racker*)
1 unit (*Racker*) = 2073 units (*Wróblewski*).

Proceeding directly from the measured values:
for measurements at 340 mμ:

(ΔE/min.) × 1000 = units (*Wróblewski*)/ml. serum
(ΔE/min.) × 26.5 = units (*Bücher*)/ml. serum
(ΔE/min.) × 0.48 = units (*Racker*)/ml. serum

for measurements at 366 mμ:

(ΔE/min.) × 1890 = units (*Wróblewski*)/ml. serum
(ΔE/min.) × 50.1 = units (*Bücher*)/ml. serum
(ΔE/min.) × 0.91 = units (*Racker*)/ml. serum.

Optimum Method

Reagents

As p. 847, but because of the limited solubility of DL-alanine substitute L-alanine.

Preparation of Solutions

I. Phosphate-alanine solution (0.1 M phosphate buffer pH 7.6; 1.3 M L-alanine):
Dissolve 0.2 g. KH_2PO_4, 1.5 g. K_2HPO_4 and 11.6 g. L-alanine in doubly distilled water and make up to 100 ml.

II. α-Oxoglutarate (0.9 M):
Dissolve 510 mg. Na-α-oxoglutarate or 395 mg. α-oxoglutaric acid in *ca.* 2 ml. doubly distilled water, adjust to pH 7.4 with 1 N NaOH and dilute to 3 ml. with doubly distilled water.

III. and IV as p. 848.

Procedure

Spectrophotometric measurements

Serum and blank cuvette, see p. 848.

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

Pipette successively into the cuvette:

2.30 ml. phosphate-alanine solution (I)
0.04 ml. DPNH solution (III)
0.04 ml. LDH solution (IV)
0.50 ml. serum.

Mix, equilibrate for 5—10 min. and mix in

0.10 ml. α-oxoglutarate solution (II).

Then proceed as described on p. 848.

Calculations

Since only 0.5 ml. serum is used, then (see p. 849):

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

The GPT values measured with this method are about 2 to 2.5 times higher than those measured with the conventional method.

Stability of the Enzyme in the Serum Sample

According to[11] the GPT activity in serum decreases by about 15% after 24 hours at room temperature, by about 11% at 4°C and by about 10% at −20°C.

Sources of Error

Animal organs, especially liver, kidney and brain, are rich in glutamic dehydrogenase[12]. In spite of the use of ammonia-free reagents it is necessary to determine the extent of interference from this source. The phosphate buffer is prepared with α-oxoglutarate instead of alanine and the GPT reaction is started with alanine after measurement of the glutamic dehydrogenase activity. The difference in the ΔE/min. values is taken as the basis for the calculation of the GPT activity.

Details for Measurements in Tissue

GPT belongs to the enzymes whose activity is only partially recovered in the supernatant of a liver homogenate after centrifuging at high speed. With human liver homogenates 96–100% of the activity is found in the supernatant, while with rat liver homogenates only 65–70% is found[13]. Depending on the results of preliminary experiments, the GPT activity is either measured in the whole homogenate or in the supernatant.

The optimum conditions for human serum are not necessarily valid for sera from other species or from other organs; they may vary considerably.

Colorimetric Determination with 2,4-Dinitrophenylhydrazine

Like the GOT activity, GPT activity can be measured colorimetrically by the use of two points, because the course of the reaction is linear (see p. 849). The first colorimetric method was described by *Green et al.*[3] and involved determination of the pyruvate formed. In the most successful methods pyruvate is determined with 2,4-dinitrophenylhydrazine[14,15]. The method described here is essentially that of *Reitman* and *Frankel*[14].

Principle

After a fixed time the pyruvate formed from L-alanine and α-oxoglutarate according to equation (1), p. 846, is determined colorimetrically by treating the 2,4-dinitrophenylhydrazone with alkali. The residual α-oxoglutarate also forms a dinitrophenylhydrazone, but its absorption maximum in alkaline solution is different to that of the pyruvate hydrazone (refer to Fig. 2 on p. 843). In order to keep the contribution of the α-oxoglutarate hydrazone to the colour low, the measurements are made between 500 and 550 mμ instead of at the absorption maximum of the pyruvate hydrazone.

The method is either standardized by direct comparison with the determination of GPT with LDH as indicator enzyme (see above) or (in routine work) with standards containing different amounts of pyruvate, but with a constant molecular amount of pyruvate + α-oxoglutarate. The standard curves (GPT activity against optical density) are non-linear.

Optimum Conditions for Measurements

The principle of the method requires that a relatively low concentration of α-oxoglutarate is used (1.7×10^{-3} M instead of 3×10^{-2} M and 6.6×10^{-3} M with the optimum and conventional spectrophotometric assays respectively). The conditions of the assay are therefore not optimum. This error can only be eliminated if the method is standardized by direct comparison with the enzymatic determination of GPT with LDH as indicator enzyme (see above). Standard curves prepared with pyruvate-α-oxoglutarate do not overcome this error.

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11) *H. Südhof* and *E. Wötzel*, Klin. Wschr. *38* 1165 [1960].
12) *E. Schmidt*, *F. W. Schmidt* and *E. Wildhirt*, Klin. Wschr. *36*, 172 [1958].
13) *E. Schmidt*, *F. W. Schmidt* and *E. Wildhirt*, Klin. Wschr. *37*, 1221 [1959].
14) *S. Reitman* and *S. Frankel*, Amer. J. clin. Pathol. *28*, 56 [1957].
15) *F. Wróblewski* and *P. Cabaud*, Amer. J. clin. Pathol. *27*, 235 [1957].

Reagents *)

1. Potassium dihydrogen phosphate, KH_2PO_4, A. R.
2. Dipotassium hydrogen phosphate, K_2HPO_4, A. R.
3. DL-Alanine
4. α-Oxoglutaric 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.

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

I. Substrate-buffer solution (0.1 M phosphate, pH 7.4; 0.2 M DL-alanine; 2×10^{-3} M α-oxoglutaric acid):
Dissolve 1.50 g. K_2HPO_4, 0.20 g. KH_2PO_4, 0.030 g. α-oxoglutaric acid and 1.78 g. DL-alanine in doubly distilled water and make up to 100 ml. Check the pH (glass electrode).

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.

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 sodium hydroxide and ketone reagent are stable indefinitely if well stoppered. Store the substrate-buffer and pyruvate solution at 0—4° C. As long as bacterial growth is prevented (by addition of a few drops of chloroform), the solutions are stable for longer than a month.

Procedure

Only use 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.

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, incubate for exactly 30 min.	Do not incubate
1 ml. ketone reagent (II)	1 ml. ketone reagent (II)
—	0.2 ml. serum

Allow to stand for 20 min. at room temperature. Add to the experimental and blank tubes
10 ml. NaOH (solution III),

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

mix, after 5 min. pour into cuvettes and read the optical density against the blank. With results of over 150 units repeat the measurements with serum diluted 1 to 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	0.1	0.9
3	0.2	0.8
4	0.3	0.7
5	0.4	0.6
6	0.5	0.5

Pipette into each tube:

0.2 ml. water

1.0 ml. ketone reagent (solution II).

Mix and allow to stand for 20 min. at room temperature. Pipette into each tube

10.0 ml. NaOH (solution III),

mix and after 5 min. read the optical density against tube No. 1. Plot the optical densities (ordinate) against the GPT units (abscissa). The following relationship was found by direct comparison with the spectrophotometric method (lactic dehydrogenase as indicator enzyme):

Test tube No. 2 ≙ 27 GPT units*)/ml. serum
Test tube No. 3 ≙ 57 GPT units/ml. serum
Test tube No. 4 ≙ 95 GPT units/ml. serum
Test tube No. 5 ≙ 137 GPT units/ml. serum
Test tube No. 6 ≙ 205 GPT units/ml. serum

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

Optical density 546 mμ	GPT units*)/ml. serum	Optical density 546 mμ	GPT units*)/ml. serum
0.025	5	0.225	71
0.050	11	0.250	82
0.075	18	0.275	95
0.100	25	0.300	108
0.125	34	0.325	123
0.150	42	0.350	141
0.175	51	0.375	160
0.200	60		

Calculations

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

*) Units according to *Wróblewski*[2].