A Kinetic Photometric Method for Serum γ-Glutamyl Transpeptidase

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A kinetic method is described for the determination of γ-glutamyl transpeptidase activity in serum. Optimal conditions for the reaction were ascertained, normal values in milliunits per milliliter at 25° established, and the reliability of the method examined. γ-Glutamyl-p-nitroanilide in ammediol-HCl buffer at pH 8.2 is employed as substrate. The method requires only 0.1 ml. of serum and, using an automatic cuvet positioner and recorder as well as an automatic diluter, more than 60 determinations can be carried out in 1 hr. On the basis of a large number of determinations, the method was shown to be technically excellent and clinically valuable.

A RISE IN SERUM γ-GLUTAMYL TRANSPEPTIDASE (γ-GT) ACTIVITY is observed almost exclusively in diseases of the liver, bile ducts, and pancreas. Extremely high values generally indicate cholestasis without, however, permitting further differentiation. In anicteric patients determination of γ-GT activity, particularly as a screening test, has proved to be very valuable (*1–4*).

In hepatobiliary diseases the serum activity of alkaline phosphatase, leucine aminopeptidase, and γ-GT are equally subject to change, with the last being by far the most sensitive (*5–8*).

Glutathione was used originally as substrate for the determination of γ-GT activity (*9–12*) .This was later replaced by γ-glutamylnaphthylamide (*13*) and γ-glutamylanilide (*14*). The aromatic compounds set free during transpeptidation were diazotized and coupled immediately with a chromophore to produce an azo dye.

A marked simplification results from using γ-glutamyl-p-nitroanilide as substrate. Unlike the cleavage product p-nitroaniline, this substrate

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From the Laboratory Department, City Hospital, Frankfurt a.M.-Höchst, Germany.

Supported by Boehringer, Ltd., Mannheim.

Presented in part at the Meeting for Biochemical Analysis, Munich, Germany, May 1, 1968.

The author is indebted to Misses E. Kinne, U. Hardt, and H. Kölbach for their excellent technical assistance.

Received for publication Mar. 16, 1968; accepted for publication June 21, 1968.

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does not absorb at 405 nm (*15, 16*), so a direct kinetic determination is possible.

In this paper a kinetic method is described using γ-glutamyl-p-nitroanilide, and the optimal reaction conditions are established. Furthermore, the reliability of the method is tested and the normal range ascertained, in milliunits per milliliter at 25°, for human serum.

Materials and Method

Apparatus

An Eppendorf photometer with an automatic recording device, an automatic cuvet positioner (temperature-controlled cuvet holder for six cuvets), and a linear absorbance converter,* as well as an automatic diluter (test-reagent-dose unit 5230),* or Eppendorf micropipets* and electric stirrer (Mikromix)* were used.

Reagents

1. Ammediol-(2-amino-2-methyl-propane-1,3-diol)-HCl buffer, 0.05 M, pH 8.6 (Schuchardt, Munich) Dissolve 526 mg. ammediol in about 80 ml. double-distilled water, adjust the pH exactly to 8.6 with 1.0 N HCl (about 2 ml.), and make up to 100 ml. with double-distilled water.

2. Substrate L-γ-Glutamyl-p-nitroanilide,† 4.4 mM; glycylglycine, 22.0 mM (Schuchardt, Munich); and magnesium chloride, 11.0 mM. Dissolve 126.3 mg. L-γ-glutamyl-p-nitroanilide, 290.7 mg. glycylglycine, and 223.7 mg. $MgCl_2 \cdot 6H_2O$ with constant stirring in 100 ml. of buffer (Solution 1) at 50–60°. The solution has a pH of about 8.2 at 25° and is already supersaturated, though at room temperature the substrate precipitates out only after 3–5 days.

A solution freshly prepared as above gives an absorbance of 0.4–0.6 measured against buffer at 405 nm in a 10-mm. cuvet. The intensity of the color depends upon the temperature at which the solution was prepared and increases 15–20% daily when stored at room temperature whether in the dark or in daylight.

Procedure

1. With the automatic cuvet positioner and recorder: To 0.1 ml. of serum in each of six halfmicrocuvets (10-mm. light path and 4-mm. width) is added 1 ml. buffered substrate. No additional mixing is necessary when the automatic diluter is used. When micropipets are used

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†L-γ-Glutamyl-p-nitroanilide was synthesized by Boehringer Ltd., Mannheim, Germany, and generously was placed at our disposal.

the solution is mixed with the electric stirrer. The automatic cuvet positioner and the paper feed in the recorder are now started. With a chart speed of 2 cm./min., the cuvets remain in the light path for 15 sec. each. The process is repeated twice. The increase in absorbance is measured at 405 nm at 25°.

2. Direct reading: For each test the increase in absorbance (ΔA) is read every 1–2 min. for 5 min., and the average per minute is calculated.

Calculation

With the semi-automatic instrument: The activity is calculated from the angle (α) formed between the time axis and the time-conversion-rate line (*17, 18*); it is expressed in milliunits per milliliter (mU./ml.).

$$1 \text{ U.} = \frac{1 \ \mu\text{Mol substrate transformed}}{1 \text{ min.}}$$

$$1 \text{ mU./ml.} = 0.001 \text{ U. (International unit)}$$

$$1 \text{ mU./ml.} = tg\alpha \times C$$

The calibration constant (C) is calculated from the following formula:

$$C = \frac{10^6}{\epsilon} \times \frac{TV}{SV} \times \frac{v}{B} = 55.6$$

where:

ϵ (molar absorptivity of p-nitroaniline at 405 nm) = 9900 M^{-1} $cm.^{-1}$
TV (total volume) = 1.1 ml.
SV (sample volume) = 0.1 ml.
v (speed of paper chart in recorder) = 2 cm./min.
B (paper width in centimeters corresponding to a difference in absorbance of 1.0) = 40 cm.

The activity was calculated between 0 and 70° for every degree (Table 1).

Direct reading:

$$1 \text{ mU./ml.} = \Delta\text{A/min.} \times \frac{10^6}{\epsilon} \times \frac{TV}{SV} = \Delta\text{A/min.} \times 1111$$

Experimental Results

pH

The dependence of γ-GT activity upon pH was tested for eight serums (15–260 mU./ml.) in Tris-HCl or ammediol-HCl buffer in the pH range 7.3–8.6 or 7.8–9.5, respectively. Between 8.0 and 8.2, we obtained a relatively broad pH optimum (Fig. 1). The curve was identical in both buffers. The concentration of ammediol did not influence the reaction rate in the range 0.01–0.2 M at constant substrate and glycylglycine concentrations. γ-Glutamyl-p-nitroanilide, however, is less soluble at ammediol concentrations greater than 0.05 M.

With a rise in pH, spontaneous substrate hydrolysis increases slightly

Table 1. Determination of γ-Glutamyl Transpeptidase Activity from the Angle (α) Formed Between Time Axis and Time-Conversion-Rate Line

α	*Activity (mU./ml.)*	α	*Activity (mU./ml.)*	α	*Activity (mU./ml.)*	α	*Activity (mU./ml)*
1	1.0	19	19.1	36	40.4	54	76.5
2	2.0	20	20.2	37	41.9	55	79.4
3	2.9	21	21.4	38	43.4	56	82.5
4	3.9	22	22.5	39	45.0	57	85.6
5	4.8	23	23.6	40	46.7	58	89.0
6	5.8	24	24.7	41	48.3	59	92.5
7	6.8	25	25.9	42	50.0	60	96.3
8	7.8	26	27.1	43	51.8	61	100.3
9	8.8	27	28.4	44	53.7	62	104.6
10	9.8	28	29.6	45	55.6	63	109.1
11	10.8	29	30.8	46	57.6	64	114.0
12	11.8	30	32.1	47	59.6	65	119.2
13	12.8	31	33.4	48	61.7	66	124.9
14	13.8	32	34.8	49	63.9	67	131.0
15	14.9	33	36.1	50	66.3	68	137.6
16	16.0	34	37.5	51	68.7	69	144.8
17	17.0	35	38.9	52	71.2	70	152.7
18	18.1			53	73.8		

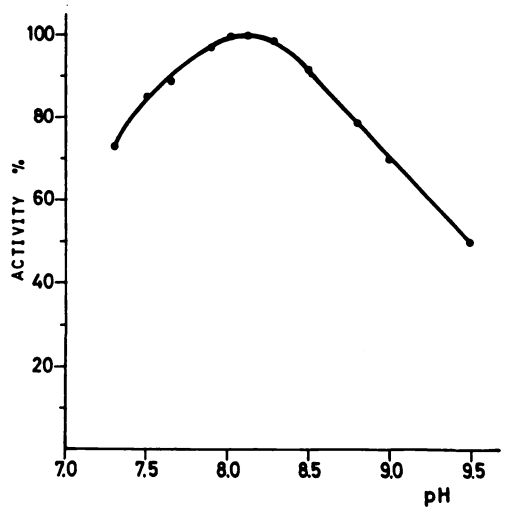


Fig. 1. Dependence of γ-glutamyl transpeptidase activity upon pH.

though distinctly. In ammediol it is equivalent to about 1.0 mU./ml. at pH 8.2 and to about 2.5 mU./ml. at pH 9.5.

Substrate Concentration

The solubility of the substrate in different buffers decreases in the following order: ammediol, diethanolamine, Tris, and triethanolamine. A concentration of 6.6 mM (i.e., 6.0 mM final concentration) can still be reached in all four buffers for use within 1–2 hr.

The enzyme activity was the same in all four buffers. The optimum occurs at 4 mM substrate concentration; between 4.0 and 6.0 mM, no further increase in activity could be detected (Fig. 2). Likewise, spontaneous substrate hydrolysis is dependent upon substrate concentration and, on the average, equivalent to 1.0 mU./ml. at 4.0 mM, and to 2.0 mU./ml. at 6.0 mM.

Graphic calculation of the Michaelis constant in serum according to Lineweaver and Burk (Fig. 3) showed good agreement for 3 serums with varying activities. As a result, the Michaelis constant of γ-GT in human serum for γ-glutamyl-p-nitroanilide is given by $K_m = 0.96$ mM.

Glycylglycine Concentration

The dependence of the γ-GT activity upon the concentration of glycylglycine as acceptor for the liberated glutamyl residue is illustrated in Fig. 4. Without glycylglycine, the role of acceptor either is taken over by the substrate itself (*15*) or else hydrolysis takes place (*14*). With a glycylglycine concentration of less than 5.0 mM, we observed competition between the various reactions; at higher concentrations, however, the side reactions are repressed in favor of the transfer of the γ-glutamyl residue to glycylglycine. The optimum is reached only at a concentration of 50 mM. Since at a glycylglycine concentration of 30 mM a precipitate forms usually after 24 hr., we work routinely with a concentration of 20 mM. This concentration is found to give, on the average, 82% of the activity obtained wih 50 mM glycylglycine concentration; in the absence of an acceptor, only about 10% of the activity at optimal concentration is found.

The Michaelis constant of human serum γ-GT for glycylglycine, calculated according to Lineweaver and Burk, for the range 5–50 mM glycylglycine concentration (Fig. 5) is $K_m = 6.65$ mM.

Other amino acids and peptides also can act as acceptor. The relative figures obtained by us for glycine (20 mM) and glycylglycylglycine (20 mM) were 17 and 20%, respectively, taking glycylglycine (20 mM) as 100%.

Activation

Addition of $MgCl_2$ at final concentrations of 2.5, 5.0, 10.0, and 20.0 mM did not alter the γ-GT activity. Even in 24 serums with very high activity (147–982 mU./ml.), the reaction rate was not raised by addition of $MgCl_2$. Likewise, preincubation at 25° with EDTA (10 mM) for 1 hr. had no effect on the activity.

For the definite clarification of the question whether Mg^{++} has an influence on the cleavage of γ-glutamyl-p-nitroanilide by human serum

γ-GT, two pooled serums (17.4 and 71.2 mU./ml., respectively) were dialyzed against EDTA (10 mM in 0.05 M ammediol buffer, pH 8.2) for 24 hr. at room temperature. Immediately thereafter, the dialysis was continued against buffer for 2 hr.; after changing the buffer, the serum

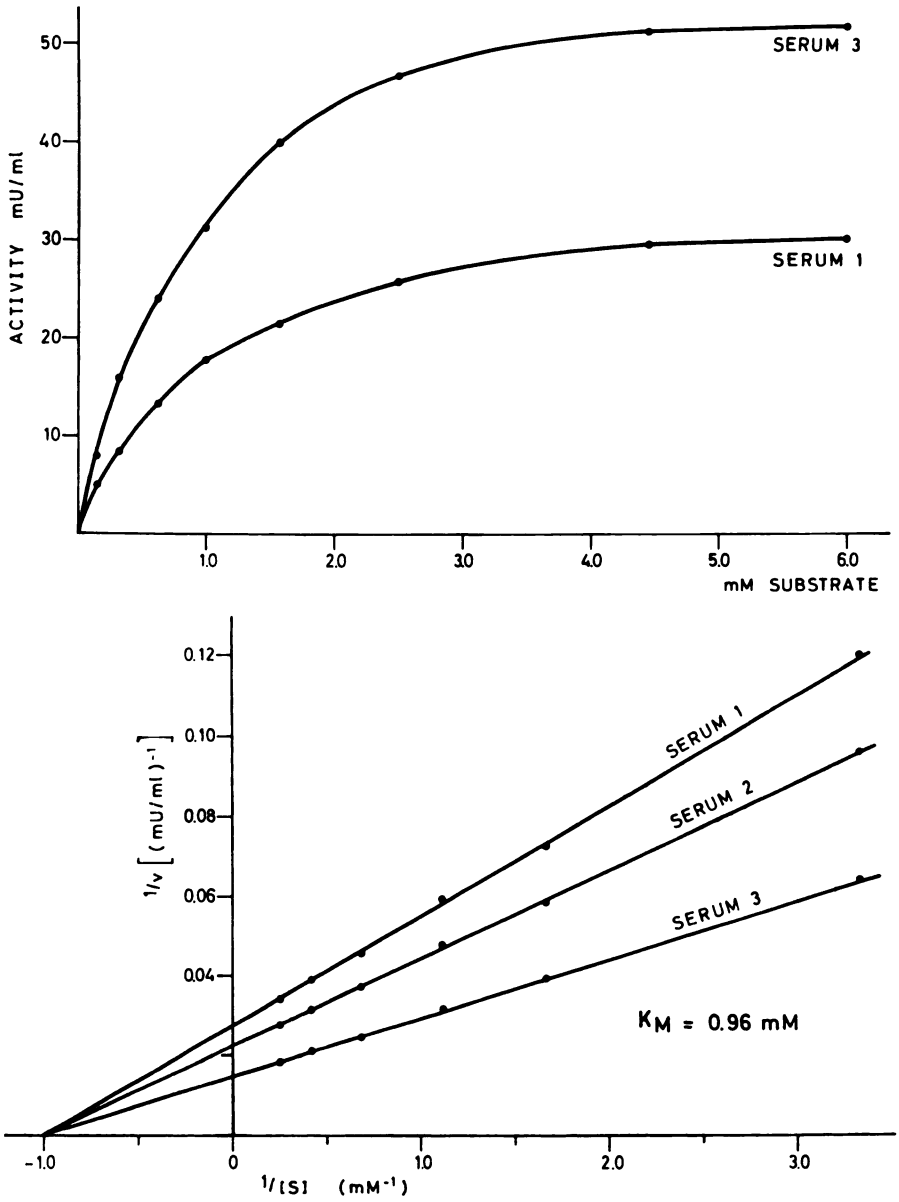
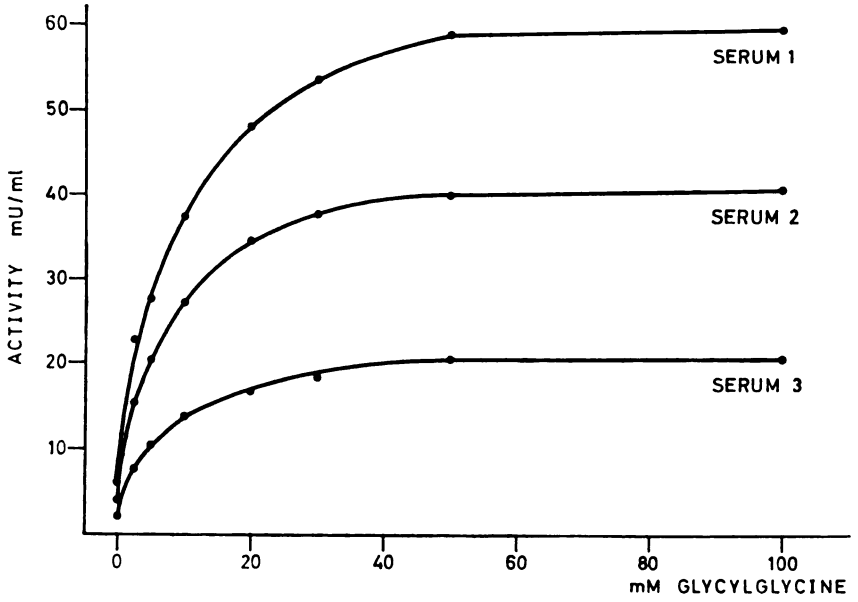


Fig. 2 (*top*). Dependence of γ-glutamyl transpeptidase activity upon concentration of γ-glutamyl-p-nitroanilide at constant glycylglycine concentration (20 mM). **Fig. 3** (*bottom*). Evaluation of Michaelis constant of human serum γ-glutamyl transpeptidase for γ-glutamyl-p-nitroanilide in 0.05 M ammediol-HCl buffer pH 8.2, according to Lineweaver-Burk.



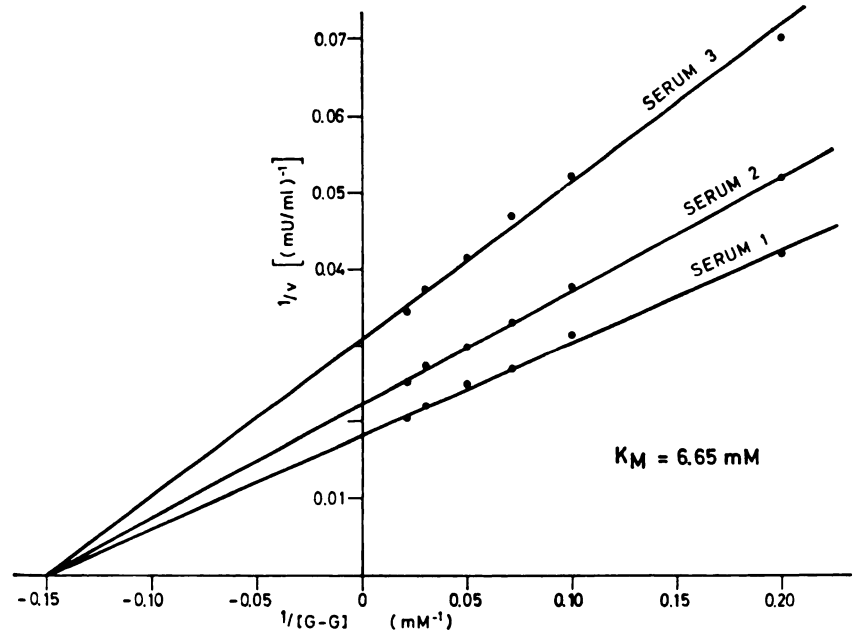


Fig. 4 (*top*). Dependence of γ-glutamyl transpeptidase activity on glycylglycine concentration at constant γ-glutamyl-p-nitroanilide concenetration (4 mM). **Fig. 4** (*bottom*). Evaluation of Michaelis constant of human serum γ-glutamyl-transpeptidase for glycylglycine in 0.05 M ammediol-HCl buffer pH 8.2 according to Lineweaver-Burk.

pool was further dialyzed for 12 hr. The same serums dialyzed exclusively against buffer, as well as the remainder of both serums kept at room temperature, served for comparison.

The activities, based on the total protein content, did not differ from one another whether pretreated or not. As the presence of $MgCl_2$ seems to promote the solubility of the substrate, the buffer substrate mixtures routinely contain $MgCl_2$ at a concentration of 10 mM.

Enzyme Concentration

The relationship between enzyme concentration (i.e., amount of serum) and enzyme activity remained linear within the range of 0–134.0 mU./ml. (Fig. 6).

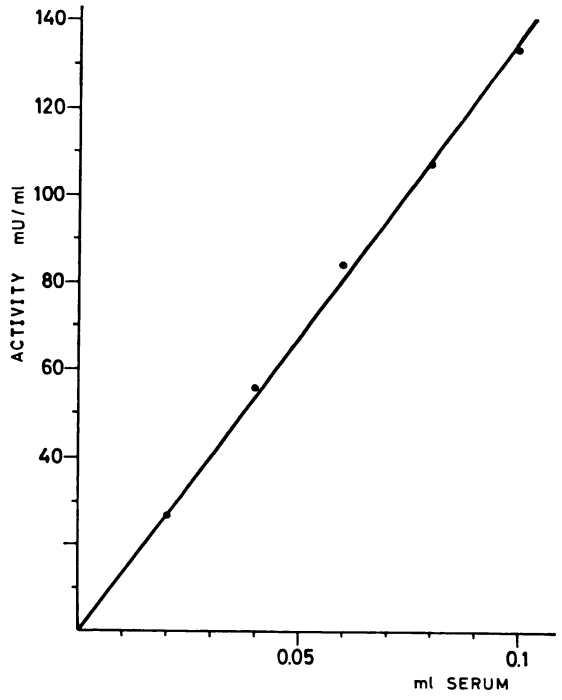


Fig. 6. Effect of enzyme concentration on γ-glutamyl transpeptidase activity.

Incubation Time

The course of the reaction was recorded continuously for serum with an activity of 103 mU./ml. During the first 10 min. the rate of reaction was completely linear, and after 20 min. 90% of the initial activity was exhibited.

With a substrate concentration of 4.0 mM, this serum transformed, in 10 min., about 2.3% (0.1 μmoles) of substrate originally present (4.4 μmoles).

Temperature

The activity rises with an increase in the temperature of incubation up to 42–45°; at higher temperatures it falls again, and above 60° no activity can be found. Autolysis also is increased appreciably at higher temperatures (Fig. 7).

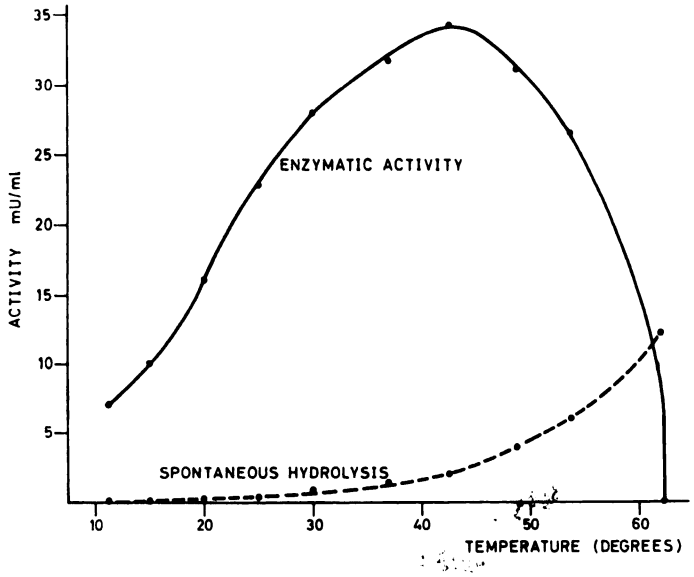


Fig. 7. Dependence of enzymatic transpeptidation and autolysis of γ-glutamyl-p-nitroanilide upon temperature of incubation.

Only in the range 11–25° does a linear relationship exist between the logarithms of the initial activities (log v_0) and the reciprocals of the absolute temperatures (1/T); beyond this we found a definite tendency downward (Fig. 8).

Accordingly, we derived the activation energy (E) only for the range 11–25°. The E value calculated from the Arrhenius equation was 14,500 cal. The temperature coefficient (Q_{10}) for this temperature was 2.33. Between 25 and 37°, however, we obtained a value of only 1.50 for Q_{10}.

Stability of Enzyme

Four serums with activities of 5.6, 11.3, 33.5, and 36.1 mU./ml., respectively, were stored at −20°, 4°, and 20°. The activities of these serums were determined daily for a week. During this time no significant loss in activity could be detected.

Hemolysis

The γ-GT activity of 12 serums was determined before and after mechanical hemolysis of the erythrocytes. After hemolysis the serums

showed a hemoglobin content of 30–550 mg./100 ml. We found no significant difference between the two tests.

Bromsulphalein

In 5 patients the γ-GT activity was determined before and 3 min. after giving Bromsulphalein (BSP) intravenously. BSP concentration

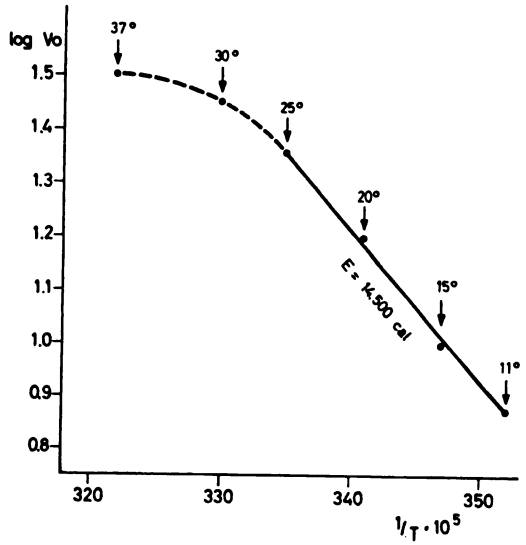


Fig. 8. Dependence of γ-glutamyl transpeptidase activity upon temperature of incubation—plotted according to Arrhenius.

was between 9.2 and 11.3 mg./100 ml. (i.e., 0.10–0.15 mM). No significant inhibition of the activity by BSP could be observed.

Reliability of Method

Specificity

γ-GT catalyzes the transfer of the γ-glutamyl group from a γ-glutamyl peptide to an amino acid or another peptide. The specificity is restricted largely to the γ-glutamyl part; the remaining portion of the peptide or molecule influences only the rate of reaction (*19*).

In the absence of an acceptor, there occurs either transpeptidation between two substrate molecules (*15*) or hydrolysis (*14*). Apart from the relative concentrations, the course of the reaction is also influenced by pH, a higher pH promoting transpeptidation (*20*).

Accuracy

Two serums with normal (14.2 mU./ml.) and elevated (52.0 mU./ml.) γ-GT activity were mixed with one another in five different proportions, and the correlation determined between the calculated activity and that actually found. A coefficient of correlation of 0.98 was obtained.

Precision

Using two pooled serums, 11 individual determinations were carried out by each of three technicians with the automatic diluter, as well as with the micropipets. The results are summarized in Table 2.

Normal Values

The control group comprised blood donors and some members of the hospital staff. Apart from γ-GT, we also determined the activity of both transaminases (*21*), alkaline phosphatase (*22*), leucine aminopeptidase (*18*), and cholinesterase (*23*). Only persons with completely normal activity of these five enzymes were included in the control group.

A total of 57 females, aged 18–61 years, and 61 males, aged 23–69 years, satisfied these conditions. The age distribution was similar in both sexes: in the range 20–50 years, the separate decades were about equally represented, and more than 80% of those examined were not over 50 years old.

On the average, the males showed a 50% higher γ-GT activity than the females. Data of males and females therefore were treated separately.

In both sexes, γ-GT values showed a log-normal distribution, so that statistical analysis was carried out on the logarithms of the data. At 25° a normal range (log $\bar{X} \pm 2$ S.D.) of 3.2–13.5 mU./ml. was obtained for females, and for males 4.5–24.8 mU./ml.

Discussion

The method described here is carried out easily. With suitable technical apparatus, at least 60 determinations can be made in 1 hr. Precision was remarkably good when the automatic diluter was employed

Table 2. Precision of the Method

	Automatic diluter			*Micropipets*		
Technician	$\bar{X}$ (*mU./ml.*)	*S.D.* (*mU./ml.*)	*C.V.* (%)	$\bar{X}$ (*mU./ml.*)	*S.D.* (*mU./ml.*)	*C.V.* (%)
Pool No. 1						
1	19.51	0.51	2.6	18.69	0.73	3.9
2	21.03	0.95	4.5	20.14	1.14	5.7
3	20.40	1.09	5.3	20.30	1.12	5.5
Pool No. 2						
1	37.94	0.78	2.1	36.81	1.39	3.8
2	40.37	0.75	1.9	38.77	1.14	3.0
3	37.88	1.26	3.3	38.65	2.09	5.4

* Each mean ($\bar{X}$) is calculated from 11 determinations.

† Coefficient of variation (C.V.) in percent = standard deviation (S.D.) ÷ $\bar{X} \times 100$.

and acceptable when micropipets were used. Principally, the automatic diluter is an improvement; comparative figures shown in Table 2 were achieved after very short practice by technicians who, at the time, had had years of experience with micropipets. Furthermore, six cuvets can be dealt with by this apparatus in 1 min., whereas it takes at least 3 min. using the micropipets.

Besides its simplicity, the γ-glutamyl-p-nitroanilide method possesses another advantage: only with this substrate is it possible to work at the optimal substrate concentration. With all other substrates, although a higher concentration is used, saturation of the enzymes is not achieved (*13, 14*).

The pH optimum at 8.1–8.2 is distinctly lower than that described for γ-glutamyl naphtylamide at pH 8.8–9.0 (*13*) and for γ-glutamyl anilide at pH 8.6–8.8 (*14*). For the determination of γ-GT activity in pig kidney with γ-glutamyl-p-nitroanilide, Orlowski and Meister (*16*) used Tris buffer at pH 9.0.

Purified enzyme of pig kidney shows no activity without Mg^{++} (*24*). For γ-GT in human serum we were unable, like other authors (*3, 13*), to find any activation at all by Mg^{++}.

γ-GT activity is inhibited competitively by glutathione, and non-competitively by BSP (*3*). It could be shown that the glutathione liberated from erythrocytes of hemolytic serum is of insufficient amount to cause inhibition. In vivo, immediately following intravenous administration of BSP, the highest concentration of BSP reached is 0.1–0.2 mM, a level insufficient to influence γ-GT activity.

As has been reported previously (*3, 19*), the γ-GT activity in the serum of males is appreciably higher than that of females.

To the present time, the activity in about 3800 serums has been determined with the method described here. In this group the method proved to be technically satisfactory and clinically very valuable. We shall report elsewhere upon our clinical findings.

References

1. Szczeklik, E., Orlowski, M., Szewczuk, A., and Kolaczkowska, B., "Serum Gamma-Glutamyl Peptidase Activity as a New Enzymatic Test in Liver Diseases." In *Proceedings of the International Congress of Gastroenterology, Leyden, 1960.* Excerpta Medica Foundation, Amsterdam, 1961.
2. Szczeklik, E., Orlowski, M., and Szewczuk, A., Serum γ-glutamyl transpeptidase activity in liver disease. *Gastroenterology* **41,** 353 (1961).
3. Goldbarg, J. A., Pineda, E. P., Smith, E. E., Friedman, O. M., and Rutenburg, A. M., A method for the colorimetric determination of γ-glutamyl transpeptidase in human serum; enzymatic activity in health and disease. *Gastroenterology* **44,** 127 (1963).
4. Rutenburg, A. M., Goldbarg, J. A., and Pineda, E. P., Serum γ-glutamyl transpeptidase activity in hepatobiliary pancreatic disease. *Gastroenterology* **45,** 43 (1963).
5. Gibiński, K., Szatoń, R., and Maraszek, J., Evaluation of gamma-glutamyltranspeptidase

(GGTP) and leucylaminopeptidase (LAP) determinations in internal disease. *Gastroenterologia* **99**, 237 (1963).

6. Aronsen, K. F., Hanson, A., and Nosslin, B., The value of γ-glutamyl transpeptidase in differentiating viral hepatitis from obstructive jaundice: A statistical comparison with alkaline phosphatase. *Acta Chir. Scand.* **130**, 92 (1965).
7. Villa, L., Dioguardi, N., Agostini, A., Ideo, G., and Stabilini, R., Prognostic value of serum γ-glutamyl transpeptidase activity in liver disease. *Enzymol. Biol. Clin.* **7**, 109 (1966).
8. Lukasik, S., Richterich, R., and Colombo, J.-P., Der diagnostische Wert der alkalischen Phosphatase, der Leucinaminopeptidase und der γ-Glutamyl-transpeptidase bei Erkrankungen der Gallenwege. *Schweiz. Med. Wochschr.* **98**, 81 (1968).
9. Hanes, C. S., Hird, F. J. R., and Isherwood, F. A., Enzymatic transpeptidation involving γ-glutamyl peptides and α-amino-acyl peptides. *Biochem. J.* **51**, 25 (1952).
10. Kinoshita, J. A., and Ball, E. G., A transpeptidation reaction between glutathione and arginine, *J. Biol. Chem.* **200**, 609 (1953).
11. Hird, F. J. R., and Springell, P. H., The enzymic reaction of amino acids with glutathione. *Biochem. J.* **56**, 417 (1954).
12. Ball, E. G., Revel, J. P., and Cooper, O., The quantitative measurement of γ-glutamyl transpeptidase activity. *J. Biol. Chem.* **221**, 895 (1956).
13. Orlowski, M., and Szewszuk, A., Colorimetric determination of γ-glutamyl transpeptidase activity in human serum and tissues with synthetic substrates. *Acta Biochim. Polon.* **8**, 189 (1961).
14. Goldbarg, J. A., Friedman, O. M., Pineda, E. P., Smith, E. E., Chatterji, R., Stein, E., and Rutenburg, A. M., The colorimetric determination of γ-glutamyl transpeptidase with synthetic substrate. *Arch. Biochem. Biophys.* **91**, 61 (1960).
15. Orlowski, M., and Meister, A., γ-Glutamyl-p-nitroanilide: A new convenient substrate for determination and study of L- and D-γglutamyltranspeptidase activities. *Biochim. Biophys. Acta* **73**, 679 (1963).
16. Orlowski, M., and Meister, A., Isolation of γ-glutamyl transpeptidase from hog kidney. *J. Biol. Chem.* **240**, 338 (1965).
17. Weber, H., and Richterich, R., Eine einfache und rasche graphische Methode zur Berechnung von Enzymaktivitäten. *Klin. Wochschr.* **41**, 665 (1963).
18. Szasz, G., A kinetic photometric method for serum leucine aminopeptidase. *Am. J. Clin. Pathol.* **47**, 607 (1967).
19. Orlowski, M., and Szewczuk, A., Determination of γ-glutamyl transpeptidase activity in human serum and urine. *Clin. Chim. Acta* **7**, 755 (1962).
20. Revel, J. P., and Ball, E. G., The reaction of glutathione with amino acids and related compounds as catalyzed by glutamyl transpeptidase. *J. Biol. Chem.* **234**, 577 (1959).
21. Feissli, S., Forster, G., Laudahn, G., Schmidt, E., and Schmidt, F. W., Normal-Werte and Alterung von Hauptketten-Enzymen im Serum. *Klin. Worchschr.* **44**, 390 (1966).
22. Hausamen, T.-U., Helger, R., Rick, W., and Gross, W., Optimal conditions for the determination of serum alkaline phosphatase by a new kinetic method. *Clin. Chim. Acta* **15**, 241 (1967).
23. Szasz, G., Cholinesterase Bestimmung im Serum mit Acetyl- und Butyrylthiocholin als Substrat. *Clin. Chim. Acta* **19**, 191 (1968).
24. Binkley, F., Purification and properties of renal glutathionase. *J. Biol. Chem.* **236**, 1075 (1961).