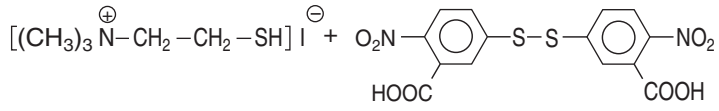
Acetylcholinesterase (AChE; Acetylcholine-Acetylhydrolase EC 3.1.1.7) and Cholinesterase (ChE; Acylcholine-Acylhydrolase EC 3.1.1.8)

Application	Determination of AChE in erythrocytes Determination of ChE in plasma
Analytical principle	Photometry
Completed in	February 1990

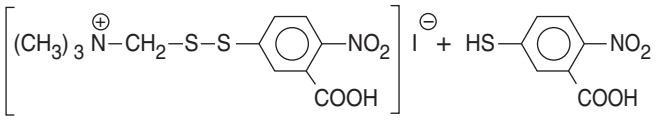
Summary

The method described here permits determination of the physiological activity of the enzymes acetylcholinesterase and cholinesterases as well as their reduced activity in persons occupationally exposed to cholinesterase inhibitors. Acetylcholinesterase (AChE) in erythrocytic membranes or cholinesterases present in plasma are assayed [1].

AChE or ChE activity is determined photometrically by means of a kinetic test after the method of *Ellmann* and *Knedel* [2, 3]. After isolation, washing and haemolysis, the erythrocytes are mixed with the substrate acetylthiocholine iodide to determine the AChE activity. For ChE determination plasma is directly mixed with the substrate. Acetylthiocholine iodide (see Section 9) is converted to acetate and thiocholine iodide by the corresponding esterase. Thiocholine iodide and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB; Ellmann's reagent) react to form the yellow dye-stuff 5-thio-2-nitrobenzoic acid as shown in the following equation:



Thiocholine iodide DTNB



5-Mercaptothiocholine-2-nitrobenzoic acid 5-Thio-2-nitrobenzoic acid

The enzyme activity is proportional to the rate of formation of the dye-stuff. Its extinction is measured at 405 nm (400–440 nm) four times after addition of the substrate and the mean extinction difference is calculated. The enzyme activity is obtained from the extinction difference.

Determination of the enzyme activity is carried out using commercially available tests in each case.

AChE activity

Within-series imprecision:	Standard deviation (rel.)	s_w = 5.8 %
	Prognostic range	u = 12.3 %
	At a mean activity of 4160 U per litre haemolysat and where n = 16 determinations	

ChE activity

Within-series imprecision:	Standard deviation (rel.)	s_w = 3.3 or 10.6 %
	Prognostic range	u = 7.5 or 22.9 %
	At activities of 1000 or 2538 U per litre plasma and where n = 10 or 14 determinations respectively	
Between-day imprecision:	Standard deviation (rel.)	s = 14.0 %
	Prognostic range	u = 31.7 %
	Using commercially prepared serum and where n = 10 days	
Inaccuracy:	A series of quality control tests was used to check the accuracy. The results lay within the range permitted by the manufacturers of the control serum (see Section 8.2).	
Detection limit:	235 U per litre	

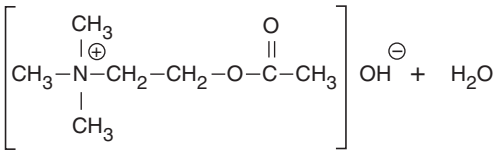
Cholinesterases

Cholinesterases hydrolyze choline esters to give choline and the corresponding carbonic acids. Two different types of cholinesterases are known: acetylcholinesterase (AChE) and cholinesterases (ChE). They occur in every organ and tissue in the human organism [4].

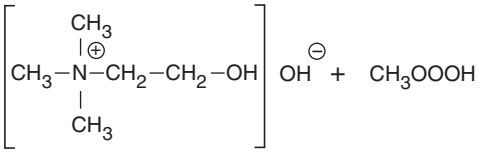
Acetylcholinesterase

Acetylcholinesterase, also called specific cholinesterase or real cholinesterase (systematic name acetylcholine-acetylhydrolase, EC 3.1.1.7) is structurally bound in membranes. It occurs in the gray matter of the central nervous system, in the cholinergic synapses (preganglionic sympathetic synapses and pre- and postganglionic parasympathetic synapses) as well as in the motor end-plates in muscles. In addition, AChE occurs in erythrocyte membranes.

The physiological and optimal substrate for AChE is acetylcholine. Acetylcholine acts as a neurotransmitter in the autonomic nervous system and the motor end-plates. The neurotransmitter is hydrolyzed and thus inactivated by AChE to give choline and acetic acid [5]:



Acetylcholine



Choline

Cholinesterases

Cholinesterases (ChE) are also known as non-specific cholinesterases, pseudocholinesterases, plasma or serum cholinesterases, butyrylcholinesterases or benzoylcholinesterases (systematic name acylcholine-acylhydrolase, EC 3.1.1.8). They occur in various

isoenzymes, their substrate specificity is lower than AChE and they are found in numerous tissues in the organism. The cholinesterases occur in plasma at a concentration of 7–9 mg/L. Their physiological function is still unknown. Among other applications they are used in pharmacology for the hydrolysis of phosphoric esters and carbamates.

Reaction mechanism and inhibition of AChE and ChE

In principle, there is no difference between the enzymatic reaction mechanism of AchE and ChE. Further details are given in Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte) und Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA) under the heading Acetylcholinesterase inhibitors [6].

In addition to their physiological substrates AChE and ChE can break down the following substances:

organophosphoric esters
carbamates
organosulfonate esters

Some of these substances can inhibit the activity of the enzyme. Inhibition due to phosphoric and sulfonate esters is irreversible, but inhibition due to carbamates is reversible. Inhibition occurs because the half life of the enzyme-inhibitor complex is considerably longer than that of the enzyme-substrate complex. When reacting with the substrate acetylcholine the original receptor structure is rapidly restored after the breakdown of the complex. In the case of carbamate poisoning a slow, but complete structural restoration takes place; in the case of poisoning with organophosphoric esters and organosulfonate esters breakdown of the enzyme-inhibitor complex is very slow and incomplete so that the enzyme must be largely replaced by renewed synthesis.

Inhibition of ChE is of no physiological importance. However, the acetylcholine metabolism of the synapses and end-plates is decisively disrupted as a result of AChE inhibition. Free acetylcholine affects the electrical potential of the nerves due to changes in the sodium and potassium ion permeability of the membranes at the nerve endings. Thus, if liberated acetylcholine cannot be rapidly broken down and inactivated by AChE spasms occur. Eventually paralysis of the striated musculature and respiratory failure can occur.

Stress and strain

Inhibition of AChE in the neurones as a result of exposure to phosphoric esters, carbamates and sulfonate esters represents the real toxicological stress parameter. However, determination of AChE in the neurones is impossible. The AChE bound to the erythrocytes is, however, the correlate of the AChE in the neurones. Thus inhibition of erythrocytic AChE is determined as a measure of the stress parameter.

Determination of ChE activity in the plasma gives an indication of possible inhibition of acetylcholinesterase. However, it is of no diagnostic significance in preventative occupational medicine. For diagnostic assessment of the measured AChE or ChE

activities it is important to know the normal enzyme activity of the individual i.e. the activity prior to exposure. As there is a relatively high interindividual fluctuation of enzyme activity, the individual reference value prior to exposure to inhibitors should be determined as early as possible (pre-employment examination = individual reference value) [6]. After exposure to cholinesterase inhibitors a further activity measurement of AChE and ChE is carried out. This activity is expressed as a percentage of the individual reference value. Activity measurements can vary by up to 30 % from the individual reference value as a result of physiological fluctuations or the circadian rhythm. Inhibition of the enzyme activity exceeding 30 % can be attributed to contact with cholinesterase inhibitors. Thus, the BAT value for acetylcholinesterase inhibitors as well as the BAT value of the organophosphate Parathion are each given as a reduction of the AChE activity to 70 % of the reference value [8]. Such results should be checked by repeated determination of the enzyme activity, for example after a recovery interval of at least 16 hours. It is advisable to choose the same time of day for renewed sampling in order to eliminate the influence of daily fluctuations. Further recommendations can be found in Arbeitsmedizinisch-toxikologische Begründungen: Acetylcholinesterase [6].

The range of fluctuation is considerably greater for ChE than for AChE. In contrast, the diagnostic sensitivity of ChE is higher, without however, any diagnostic specificity for the relevant cholinesterase inhibition of the synapses. On the other hand, determination of ChE activity can be used for the differential diagnosis of liver disease, as the biosynthesis of ChE takes place in the liver [4].

Normal values

Erythrocytic AChE activities between 2900 and 4100 U per litre of sampled blood were measured by the authors in healthy male adults using the photometric method and acetylthiocholine as a substrate (see Section 9). In the plasma of healthy test persons the authors measured the following ChE activities using acetylthiocholine as a substrate:
Women: 1700–3700 U per litre of sampled blood
Men: 1900–3800 U per litre of sampled blood

Authors: *J. Lewalter, C. Domik*
Examiner: *K.H. Schaller*

Acetylcholinesterase (AChE; Acetylcholine-Acetylhydrolase EC 3.1.1.7) and Cholinesterase (ChE; Acylcholine-Acylhydrolase EC 3.1.1.8)

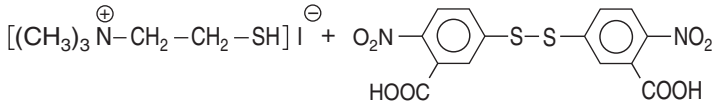
Application	Determination of AChE in erythrocytes Determination of ChE in plasma
Analytical principle	Photometry
Completed in	February 1990

Contents

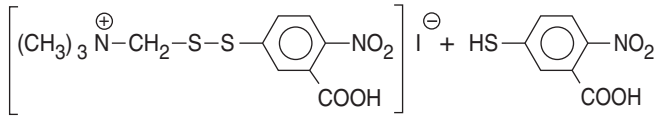
1 General principles

Acetylcholinesterase (AChE) in the erythrocyte membranes or the cholinesterases (ChE) present in plasma can be determined by the method described here [1].

AChE or ChE activity is determined photometrically by means of a kinetic test after the method of *Ellmann* and *Knedel* [2, 3]. After isolation, washing and haemolysis the erythrocytes are mixed with the substrate acetylthiocholine iodide to determine the AChE activity. For ChE determination plasma is directly mixed with the substrate. Acetylthiocholine iodide (see Section 9) is converted to acetate and thiocholine iodide by the corresponding esterase. Thiocholine iodide and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB; Ellmann's reagent) react to form the yellow dye-stuff 5-thio-2-nitrobenzoic acid as shown in the following equation:



Thiocholine iodide DTNB



5-Mercaptothiocholine-2-nitrobenzoic acid 5-Thio-2-nitrobenzoic acid

The enzyme activity is proportional to the rate of formation of the dye-stuff. Its extinction is measured at 405 nm (400–440 nm) four times after addition of the substrate and the mean extinction difference is calculated. The enzyme activity is obtained from the extinction difference.

Determination of the enzyme activity is carried out using commercially available tests in each case.

2 Equipment, chemicals and solutions

2.1 Equipment

Single or double beam spectrophotometer or filter photometer capable of measurement at 405 nm (or 400–440 nm); a kinetic program in the photometer and a cuvette holder which can be thermostatically regulated are to be recommended

Cuvettes with layer thickness of 1 cm; disposable or suction cuvettes are also suitable
Disposable syringes containing potassium EDTA as an anticoagulant (e.g. Monovetten® from Sarstedt)
Centrifuge
Centrifuge tubes (plastic) with a 1 cm graduation, contents 5 mL
2 and 3 mL Pipettes
Haematocrit centrifuge with measuring template
20 µl Microcapillaries, calibrated (e.g. from Brand)
Microlitre pipettes with adjustment of 20, 100 and 1000 µL (e.g. from Eppendorf)
Roller mixer
Vortex mixer
Stop-watch
Water bath, adjusted to 25 °C

2.2 Chemicals

5,5'-Dithio-bis-2-nitrobenzoic acid (e.g. test combination cholinesterase with acetylthiocholine as substrate from Boehringer)
Acetylthiocholine iodide (e.g. test combination cholinesterase with acetylthiocholine as substrate from Boehringer)
Ultrapure water (ASTM type 1) or double-distilled water
Isotonic saline solution (154.0 mmol/L) (e.g. from Fresenius)
Control samples for quality control (e.g. Precinorm U or Precipath U from Boehringer)

2.3 Solutions

0.26 mM 5,5'-Dithio-bis-2-nitrobenzoic acid in 52 mM phosphate buffer pH 7.2. The solution can be stored in the refrigerator for six weeks in a brown glass bottle.

156 mM Acetylthiocholine iodide in ultrapure water. The solution can be stored in the refrigerator for six weeks.

3 Specimen collection and sample preparation

In each case activity of the enzyme must be determined before coming in contact with cholinesterase inhibitors. After exposure a further determination is carried out. The result is expressed as a percentage of the individual reference value (see the introductory part of this chapter under the heading Stress and strain).

3.1 Specimen collection

Blood specimens (2 mL) are drawn from the arm vein using disposable syringes containing anticoagulant. The plasma and erythrocyte samples must be prepared within eight hours of the specimen collection. Haemolysis of the erythrocytes must be prevented.

3.2 Sample preparation

In order to ensure that the erythrocytes remain undamaged while plasma and erythrocyte samples are prepared from the blood specimens it is necessary to work rapidly in isotonic solutions. Before separation of the blood into plasma and erythrocytes the haematocrit value of the whole blood (HK 1) is determined.

Preparation of the erythrocyte samples:
2 mL physiological saline is added to 1 mL whole blood, carefully mixed and centrifuged at 1200 g for 10 min. The supernatant is quantitatively drawn off and discarded. The erythrocytes are then washed twice more with 2 mL isotonic saline solution as above, centrifuged and the actual supernatants are discarded. Finally the washed erythrocytes are filled up to 1 mL with physiological saline. The haematocrit of the erythrocyte suspension is determined (HK 2). The sample can be stored in the refrigerator in this state for up to seven days without discernible activity reduction.

The erythrocyte suspension is made up to a volume of 10 mL with ultrapure water in preparation for the activity measurement. After haemolysis samples should be processed and determined that same day.

Preparation of the plasma samples:
1 mL of whole blood is centrifuged at 1200 g for 10 min. The supernatant plasma is drawn off with a pipette, made up to 1 mL with physiological saline, transferred to a disposable tube and sealed.

The plasma sample can be stored in this state for up to seven days in the refrigerator or at room temperature without discernible activity reduction. In preparation for the activity measurement the plasma solution is filled up to 10 mL with ultrapure water and should then be determined that same day.

The erythrocyte or plasma samples are gently homogenized by rolling in the roller mixer in preparation for testing.

A reagent blank is included in each test series. Ultrapure water is used instead of haemolytic erythrocytes or plasma.

4 Instrumental parameters for photometry

Either the wavelength of the spectrophotometer is adjusted to 405 nm or an appropriate filter is used. In the case of a double beam instrument measurement against air is carried out. The extinctions are read and recorded at intervals of 30 s. An automatic recording device which plots the extinction against time is preferable.

5 Analytical determination

5.1 Enzyme determination

The temperature of the dithio-bis-nitrobenzoic acid solution (solution 1) is stabilized at 25 °C. 20 µL of the prepared sample solution (plasma or haemolytic erythrocytes) and 100 µL of the substrate solution (solution 2) are added to 3 mL of this solution (solution 1) at 25 °C. The reagent blank is treated exactly as each sample.

The following pipetting scheme serves to illustrate this:

Reactants	Sample µL	Reagent blank µL
Solution 1	3000	3000
Sample	20	–
ultrapure water	–	20
Solution 2	100	100

The solution is mixed manually. After 2 min the extinction is measured at 405 nm (1st extinction value). Simultaneously the kinetic program or, as the case may be, the stop-watch is started and three further extinction readings are taken at 30 s intervals. The mean value is calculated from the three extinction differences.

The first extinction value should not exceed 0.5. If extinction differences are too high ($\Delta E/30\ s > 0.2$) the sample should be diluted with ultrapure water.

5.2 Haematocrit determination

The haematocrit (HK) value is measured in the original blood sample (HK 1) as well as in the washed erythrocyte suspension (HK 2). For this purpose the homogenized blood sample or erythrocyte suspension is drawn into a microcapillary, sealed and treated in a haematocrit centrifuge. The length of the erythrocyte sediment serves as a measure of the haematocrit value. It is read with a measuring template.

6 Calculation of the analytical result

The mean values $\overline{\Delta E}$ for each sample (as well as the reagent blank) are obtained from the three extinction differences ΔE_{1-3}. The AChE or ChE activities (in U/L) are calculated using the following equation:

AChE activity in erythrocytes:
$b_{AChE} = \overline{\Delta E}_{ery} \cdot 10 \cdot 23460$ (see Appendix)
b_{AChE} = enzyme activity of AChE

In order to compensate for any sample loss during the preparation of the erythrocytes the value for the haemolytic erythrocytes is multiplied by the factor HK 1/HK 2.

ChE activity in plasma:
$b_{ChE} = \overline{\Delta E}_{plasma} \cdot 10 \cdot 23460$ (see Appendix)
b_{ChE} = enzyme activity of ChE

The enzyme activity of the sample solution can also be obtained from the value table of the test combination.

If necessary the activity of the reagent blank must be subtracted.

In order to assess the occupational medicine implications of exposure to AChE or ChE inhibitors the activities are expressed as a percentage of the individual reference value (see the introductory part of this chapter under the heading Stress and strain).

7 Standardization and quality control

Quality control of the analytical results is carried out as outlined in TRgA 410 of the German Arbeitsstoffverordnung (Regulation 410 of the German Code on Hazardous Working Materials) [9]. Commercially available serum is used as a standard (see Section 2.2).

8 Reliability of the method

8.1 Precision

AChE activity in erythrocytes:
The within-series imprecision was determined using haemolytic erythrocytes obtained from blood from test persons. The relative standard deviation was 5.8 %, equivalent to a prognostic range of 12.3 %, for 16 determinations at a mean activity of 4160 U/L.

ChE activity in plasma:
The within-series imprecision was determined using commercially available standard serum as well as plasma prepared from the blood of test persons. 10 determinations using Precinorm U from Boehringer, for which the ChE activity is given as 1000 (800–1200) U/L, yielded a standard deviation of 3.3 % and a prognostic range of 7.5 %. When 14 determinations were carried out at a mean activity of 2538 U/L using plasma from test persons the relative standard deviation was 10.6 % and the prognostic range was 22.6 %.

The between-day imprecision was determined using commercially available serum on ten different days. The relative standard deviation was 14.0 % and the prognostic range was 31.7%.

8.2 Accuracy

The accuracy of the analyses was tested by duplicate determination using the quality control serums Monitrol I, Monitrol II, Precinorm E, Precinorm U and Precipath E from Boehringer. Table 1 shows the expected values given by the manufacturers, the permitted range and the results obtained by the author. The accuracy of the method proved to be good. In each case the results lay within the expected range.

Additional results of round-robin experiments to determine cholinesterase in plasma are available. The analyses were carried out by the Deutsche Gesellschaft für Klinische Chemie (German Society of Clinical Chemistry). Regression analysis of 42 pairs of expected values and values obtained by the examiner gave a correlation of 0.929 (Fig.1). At present there is no commercially available control material for the determination of AChE activity in erythrocytes.

8.3 Detection limit

The minimum detectable extinction difference per 30 s is about 0.01. This is equivalent to an enzyme activity of 235 U/L.

8.4 Sources of error

Under the working conditions described here the kinetic activity of AChE or ChE can be accurately and reproducibly determined.

It is essential to maintain a constant temperature of 25 °C for the extinction measurement. Both higher and lower temperatures lead to erroneous readings. If the extinction differences are not linear the measurements should be repeated taking all the methodological parameters into account.

In order to ensure correct activity measurements the following conditions must be fulfilled:
– The extinction differences must be in the linear range.
– There must be a distinct extinction difference between two readings.

– The first extinction should not exceed 0.5. If this is the case the sample should be diluted.
– The individual extinction readings must be taken exactly on schedule.

Oxalate, Heparin and EDTA are suitable anticoagulants. In contrast, fluorides should on no account be used as anticoagulants, as they inhibit the AChE or ChE activity even at low concentrations.

Icteric and lipaemic plasma do not affect the activity determination.

Inhibition of the enzyme activity can be caused by the intake of certain medicines, e.g. Adipiodon, Cyclophosphamide, Iopan acid, Neostigmin, Pancuroniom bromide, Physostigmin and Tubocurarin chloride.

9 Discussion of the method

Both AChE in the erythrocytes and ChE in plasma can be determined using the method described here. Commercially available test combinations can be used for this purpose. The procedure is simple and can be rapidly carried out.

Determination of the AChE or ChE activity is based of the work of *Ellmann* et al. [2]. The optimal substrate for determination of ChE activity is butyrylthiocholine. However, for reasons of uniformity acetylthiocholine was recommended here for ChE as well as the AChE enzyme tests. For special investigations it is advisible to use butyryltiocholine as a substrate [3] for assaying ChE in plasma, because it allows a more sensitive determination.

This substrate is broken down twice as fast as acetylthiocholine by ChE.

A reagent blank is necessary only when acetylthiocholine is used, as this substrate is broken down autolytically in aqueous solution. In contrast, no reagent blank is necessary when butyrylthiocholine is used as a substrate.

Initially acetylcholine was used as a substrate instead of acetylthiocholine. The acetate released from the acetylcholine was determined by measurement of the pH value of the solution [10]. This method did not prove to be reliable, as the pH depends on a number of other parameters. However, a sensitive and specific determination of thiocholine iodide is achieved with dithio-bis-nitrobenzoic acid. Problems of measurement seem to preclude determination of this substrate by other instrumental analytical methods.

The method described here is noteable for its reliability. Commercially available control serums can be employed to check that the precision and accuracy of the method are good.

An internal quality control for the ChE activity in plasma is possible using commercially available control serums. The organizers of round-robin experiments also included this parameter in their program both on a national and international level. At present there is no commercially available control material for the determination of AChE activity in erythrocytes.

Investigation of the kinetics of the ChE determination shows that the enzymatic reaction is linear from about 1 min to 20 min and is dependent on the enzyme activity (cf. Fig. 2).

Samples should be processed and determined as soon as possible, as longer periods of storage lead to reduction in activity. This was investigated by taking 100 µL blood from 5 test persons. Immediate haemolysis of the samples was caused by adding 5 mL distilled water. The enzyme activity was determined immediately and after various periods of storage (at 4–8°C). The enzyme activities are shown in Table 2. The results show that a gradual reduction of the AChE or ChE activity occurs even when the samples are stored in the refrigerator.

The industrial health assessment of a AChE or ChE inhibition is carried out as described in the introductory part of this chapter under the heading Stress and strain.

Instruments used:
Filter photometer from Kone with a semi-automated pipetting station, kinetic module and a 405 nm filter.

10 References

[1] *M.Whittaker:* Cholinesterases. In: *H.-U. Bergmeyer* (ed.): Methods of Enzymatic Analysis. 3rd ed.,Vol. IV. Enzymes 2.VCH,Weinheim 1986, pp. 52–74.
[2] *G. L. Ellmann, D. K. Courtney, V Andres jr.,* and *R. A. Featherstone:* A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. *7*, 88 (1961).
[3] *M. Knedel* and *R. Böttger:* Eine kinetische Methode zur Bestimmung der Aktivität der Pseudocholinesterase (Acetylcholin-acylhydrolase 3.1.1.8). Klin. Wschr. *45,* 325 (1967).
[4] *F. T. Schuh:* Über Cholinesterasen - eine anästhesiologisch wichtige Enzymgruppe. Anästhesist *25,* 501 (1976).
[5] *L. T. Potter:* Acetylcholine, choline, acetyltransferase, acetylcholinesterase. In: *A. Lajtha* (ed.): Handbook of Neurochemistry, Vol. VI. Plenum, New York, London 1970.
[6] *J. Lewalter:* Acetylcholinesterase-Hemmer. In: *D. Henschler* and *G. Lehnert* (eds.): Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte) und Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA). Arbeitsmedizinisch-toxikologische Begründungen. Deutsche Forschungsgemeinschaft,VCH, Weinheim, 3rd issue 1986.
[7] *R. L. Lauwerys:* Industrial Chemical Exposure: Guidelines for Biological Monitoring. Biomedical Publications, Davis, California 1983, pp. 118–121.
[8] *Deutsche Forschungsgemeinschaft:* Maximum Concentrations at the Workplace and Biological Tolerance Values for Working Materials 1990. Report No. XXVI of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. VCH, Weinheim 1990.
[9] TRgA 410: Statistische Qualitätssicherung. In: Technische Regeln und Richtlinien des BMA zur Verordnung über gefährliche Stoffe. Bek. des BMA vom 3.4.1979, Bundesarbeitsblatt 6/1979, pp. 88ff.
[10] *K. M. Meinecke* and *H. Oettel:* Mikromethode zur Bestimmung der Acetylcholinesterase Aktivität in Erythrozyten und Plasma von Mensch und Tier. Arch. Toxikol. *21*, 321–332 (1966).

Authors: *J. Lewalter, C. Domik*
Examiner: *K.H. Schaller*

Appendix:

The enzyme activity is calculated according to the following equation:

$$b = \frac{\Delta E}{\Delta t \cdot \varepsilon \cdot d} \cdot \frac{V_1}{V_2}$$

where

$$b = \text{enzyme activity in } \frac{\mu mol}{min \cdot L} = U/L$$

$\Delta E/\Delta t$ extinction difference per unit time in min^{-1}

ε molar extinction coefficient
here: 1.33 $L \cdot mmol^{-1} \cdot mm^{-1}$

d thickness of the layer in mm
here: 10 mm

V_1 final volume of the test preparation in ml
here: 3.12 mL

V_2 sample volume in mL
here: 0.02 mL

Thus the enzyme activity is calculated with the following factor:

$$b = \frac{\Delta E}{0.5 \text{ min} \cdot 1.33 \text{ L} \cdot \text{mmol}^{-1} \cdot \text{mm}^{-1} \cdot 10 \text{ mm}} \cdot \frac{3.12 \text{ mL}}{0.02 \text{ mL}}$$

$$= \Delta E \cdot 23460 \frac{\mu mol}{min \cdot L}$$

In this case Boehringer assumes a molar extinction coefficient of 1.33 $L \cdot mmol^{-1} \cdot mm^{-1}$ for the calculation of the factor. In the literature [1] a molar extinction coefficient of 1.36 $L \cdot mmol^{-1} \cdot mm^{-1}$ is also given.

Table 1: Accuracy control of the cholinesterase determination.
Expected values given by the manufacturer of the quality control serum. Permitted range = expected value ± 20%; according to the guidelines of the Bundesärztekammer (German Medical Council).

Quality control	Expected value (U/L)	Permitted range (U/L)	Author's results (U/L)
Monitrol I	1530	1224–1836	1519 1572
Monitrol II	1087	870–1304	1016 1043
Precinorm E	1566	1253–1879	1334 1360
Precinorm U	1000	800–1200	1122 1096
Precipath E	872	698–1046	778 725

Table 2: Activity of cholinesterase in the haemolysat (100 µL whole blood in 5 mL distilled water).

Time of measurement	Enzyme activity (U/L) Number of test person				
	1	2	3	4	5
immediately after specimen collection	5031	4540	4049	5031	4540
after					
15 minutes	4786	4417	3804	4900	4417
30 minutes	4540	4540	3681	5031	4540
60 minutes	4663	4417	3804	4908	4540
3 hours	4775	4294	3824	4785	4294
5 hours	4663	4417	3804	4786	4540
8 hours	4417	4417	3681	4663	4417
1 day	4663	4786	3681	4417	4294
2 days	4417	4540	3681	4540	4295
5 days	4295	4295	3559	4417	3927
6 days	4295	3927	3313	4049	3927
7 days	4356	4049	3497	4172	4111
9 days	4111	3804	3313	3927	3927

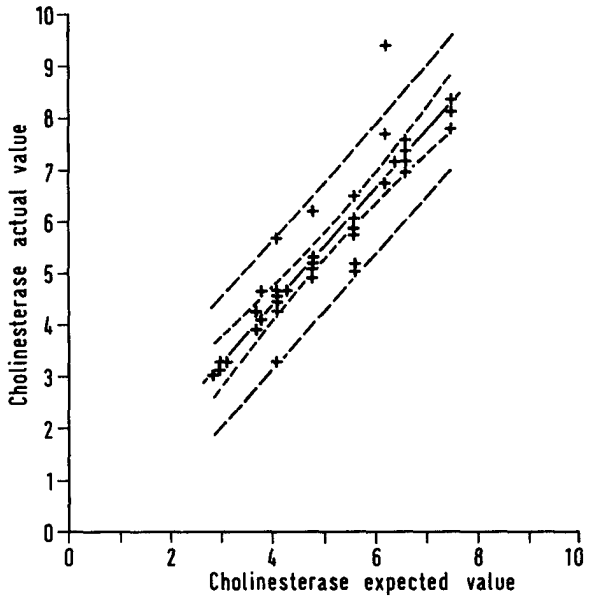


Fig. 1: Correlation diagram of actual and expected values of 42 pairs of results obtained in round-robin experiments carried out by the Deutsche Gesellschaft für Klinische Chemie (German Society of Clinical Chemistry). Correlation coefficient $r = 0.929$.

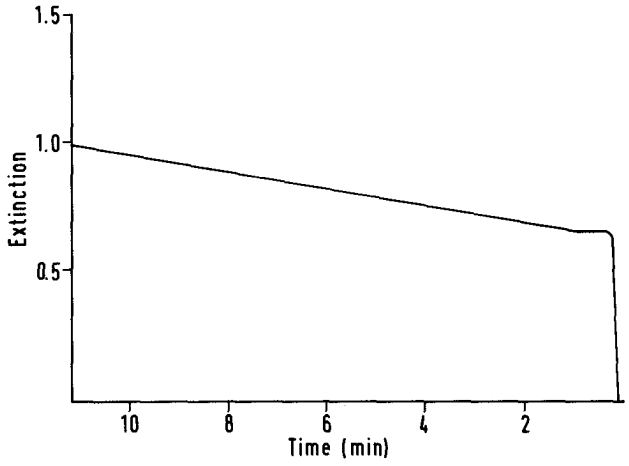


Fig. 2: Kinetics of the AChE determination.