Catalase

Hans Lück

Catalase is an enzyme present in nearly all animal and plant cells, which catalyses the following reactions:

(1) $$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

(2) $$ROOH + AH_2 \longrightarrow H_2O + ROH + A$$

It is not yet clear whether the role of catalase in the organism is to decompose hydrogen peroxide (equation 1) or to catalyse a peroxidation reaction (equation 2). Several substrates (AH_2) can be oxidized *in vitro* by catalase and H_2O_2, including methanol[1], ethanol[1,2], formic acid[2], thiols[3] and phenols[4].

Catalase combines rapidly with H_2O_2 or alkyl hydroperoxides (primary compounds). The rate constant k_1 for the reaction catalase + H_2O_2 is of the order of 10^7 sec.$^{-1} \times$ mole^{-1}. With the alkyl hydroperoxides the constant decreases with increasing chain length. In comparison to the formation of the primary compound the back reaction ($k_2 < 0.02$ sec.$^{-1}$) can be disregarded. The turnover number for the decomposition of hydrogen peroxide is 2.5×10^6 to 5×10^6 moles/min.

The usual methods for the determination of catalase activity in biological material depend on:

a) Measurements of the heat production of the catalase reaction[5]. The maximum rise in temperature after the start of the reaction can serve as an approximate measure of the activity.

b) Determination of the oxygen liberated on decomposition of H_2O_2: The manometric method[6–8] (refer to p. 29 and 40) is considerably more accurate than the volumetric measurement of oxygen with the "Kataläser"[9]. The "paper disk method" is rapid and easy to carry out, but is not so accurate[10]. In this method the enzyme activity is measured by the rate at which a filter paper disk soaked in the sample solution is carried to the surface of a H_2O_2 solution by the oxygen liberated.

c) Determination of the residual hydrogen peroxide by titration, spectrophotometry[11–13] at 230–250 mμ, by polarography[14] and electrochemically by measurements with the platinum electrode[15,16].

The spectrophotometric and titrimetric methods have proved best. In food chemistry the "Kataläser" is also used because of its simplicity.

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8) *D. Appleman*, Analytic Chem. *23*, 1627 [1951].
9) "Kataläser", see p. 891.
10) *M. Gagnon*, *W. M. Hunting* and *W. B. Esselen*, Analytic Chem. *31*, 144 [1959].
11) *R. F. Beers* and *I. W. Sizer*, J. biol. Chemistry *195*, 133 [1952].
12) *B. Chance*, J. biol. Chemistry *179*, 1299 [1949].
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15) *K. Damaschke* and *F. Tödt*, Z. Naturforsch. *11*b, 85, 621 [1956].
16) *K. Damaschke* in *F. Tödt:* Elektrochemische Sauerstoffmessungen. W. de Gruyter, Berlin 1958, p. 159.

Optimum Conditions for Measurements

With very short reaction times and relatively high enzyme concentrations the decrease of the H_2O_2 concentration with time due to the action of catalase corresponds to a first order reaction:

$$k = \frac{1}{\Delta t} \ln \frac{S_1}{S_2} = \frac{2.3}{\Delta t} \log \frac{S_1}{S_2} \quad (3)$$

where $\Delta t = t_2 - t_1$ = the measured reaction time

S_1 and S_2 = H_2O_2 concentrations at times t_1 and t_2.

Equation (3) is not entirely valid for long reaction times and low enzyme concentrations, because catalase in dilute solution is unstable and is also destroyed by hydrogen peroxide. This destruction can be minimised by working at a lower temperature (0 to +4° C). The temperature coefficient for the decomposition of H_2O_2 is about 5% per degree between 0 and +10° C. The reaction times should not exceed a few minutes and the H_2O_2 concentration should be about 0.01 M. The optimum pH of the reaction is around 7.0. Above pH 10 catalase decomposes; the molecule splitting into smaller units. At lower pH values the activity decreases sharply. Figure 1 shows the dependence on pH of the catalase spectrum, of the maximum amount of H_2O_2 hydrolysed (refer to p. 893) and of the rate constant k (equation 3) with very dilute enzyme solutions. With high concentrations of enzyme and short reaction times k is only 50% lower at pH 3 than at pH 7[17].

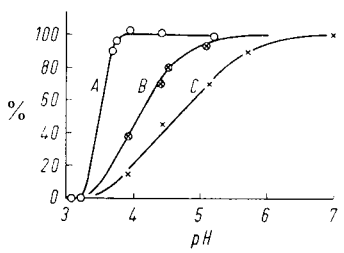

Fig. 1. Dependence on pH of the catalase activity. A) change in intensity of the Soret band (410 mμ); B) maximum amount of H_2O_2 decomposed; C) reaction rate constant k with very dilute enzyme solutions[17a].

A. Spectrophotometric Method[11–13]

Principle

The ultraviolet absorption of a H_2O_2 solution can be easily measured between 230 and 250 mμ. On decomposition of H_2O_2 with catalase the absorption decreases with time and from this decrease the enzyme activity can be calculated. This method can only be used with enzyme solutions which do not absorb strongly at 230–250 mμ.

Reagents

1. Potassium dihydrogen phosphate, A. R., KH_2PO_4
2. Disodium hydrogen phosphate, A. R., $Na_2HPO_4 \cdot 2\,H_2O$
3. Hydrogen peroxide*), *ca.* 30% (w/v)

Preparations of Solutions

I. Phosphate buffer (M/15; pH 7.0):
Dissolve 3.522 g. KH_2PO_4 and 7.268 g. $Na_2HPO_4 \cdot 2\,H_2O$ in doubly distilled water and make up to 1000 ml.

*) *e.g.* "Perhydrol", Merck.

17) *B. Chance*, J. biol. Chemistry *194*, 471 [1957].

17a) *H. Lück*, Naturwissenschaften *44*, 423 [1957].

II. H_2O_2-phosphate buffer (M/15 phosphate; *ca.* 1.25 10^{-2} M H_2O_2; pH 7):
Dilute 0.16 ml. hydrogen peroxide (30% w/v) to 100 ml. with buffer (solution I). The optical density of this solution should be about 0.500 at 240 mμ and with a 1 cm. light path.

Stability of the solutions

The phosphate buffer is stable indefinitely if bacterial contamination is avoided. Prepare the buffered hydrogen peroxide solution freshly each day.

Procedure

Experimental material

The enzyme must be in solution or suspended homogeneously for precise measurements.

Tissue: Homogenize plant or animal tissue in a blendor (see p. 50) with M/150 phosphate buffer (solution I; diluted 1:10) at 1 to 4°C and centrifuge. Stir the sediment with cold phosphate buffer, allow to stand in the cold with occasional shaking and then repeat the extraction once or twice. The extraction should not take longer than 24 hours. Use the combined supernatants (sometimes opalescent) for the assay. Section very small amounts of tissue on a freezing microtome, sufficiently thinly (20—50 μ) so that practically every cell is damaged; the cell contents can then be extracted easily and quantitatively. This method is more suited to quantitative studies than grinding the tissue in a mortar with sand or powdered glass. Extract tissue slices (600—800 mg.) two or three times with 5 to 10 ml. phosphate buffer each time as described above.

The catalase activity can change considerably on storage of the tissue. In comparative studies therefore always use the same conditions of extraction, storage and temperature.

Foodstuffs: The catalase activity of foodstuffs is usually measured titrimetrically or with the "Kataläser" (see p. 717 and 891).

Spectrophotometric measurements

Wavelength: 240 mμ; silica cuvettes, light path: 1 cm.; final volume: *ca.* 3 ml.; room temperature. Keep constant for a series of measurements or use a constant temperature cuvette chamber. Read against a control cuvette containing enzyme solution as in the experimental cuvette, but containing H_2O_2-free phosphate buffer (solution I).

Pipette into the experimental cuvette:

3.00 ml. H_2O_2-phosphate buffer (solution II)

mix in

0.01—0.04 ml. sample

with a glass or plastic rod flattened at one end. Note the time Δt required for a decrease in the optical density of from 0.450 to 0.400; this value is used for the calculations. If Δt is longer than 60 sec. repeat the measurements with a more concentrated solution of the sample.

Calculations

From equation (3) it follows that for a decrease in optical density from 0.450 to 0.400:

$$(3a) \qquad k = \frac{2.3}{\Delta t} \log \frac{E_{initial}}{E_{final}} = \frac{2.3}{\Delta t} \log \frac{0.450}{0.400} = \frac{2.3}{\Delta t} 0.05115 = \frac{0.1175}{\Delta t} [sec.^{-1}]$$

where E = optical density at 240 or 250 mμ
2.3 = factor to convert from ln to log.

The value k can be converted to any catalase units.

According to[13] a unit is the amount of enzyme which liberates half the peroxide oxygen from a hydrogen peroxide solution of any concentration in 100 sec. at 25°C. The unit of enzyme activity is therefore related to the half-life time τ of a first order reaction.

For τ the following formula is usually valid: $\tau = \frac{\ln 2}{k} = \frac{0.693}{k}$. The relationship between the observed half-life time $\tau_{observed}$ and the enzyme activity is:

(4) $$1 \text{ unit} = \frac{100}{\tau_{observed}} = \frac{k_{observed}}{6.93 \times 10^{-3}}$$

or referring to equation (3a)

(4a) $$\frac{0.1175}{\Delta t \times 6.93 \times 10^{-3}} = \frac{17}{\Delta t} = \text{units/assay mixture}$$

Example

Rat liver (1 g.) was homogenized in a blendor with phosphate buffer and extracted (see above); total volume v of extract was 20 ml. Immediately before the measurements a sample was diluted 1:10 with water and 0.01 ml. was taken for the assay. The optical density at 240 mμ decreased from 0.450 to 0.400 in 13.9 sec.

$$\frac{17}{13.9} = 1.22 \text{ units in the assay mixture}$$

or $1.22 \times 10/0.01 = 1220$ units/ml. extract, *i.e.* 2.44×10^4 units/g. liver.

B. Determination by Iodometric Titration[18]

After stopping the reaction with H_2SO_4 the residual H_2O_2 can be determined by:

a) oxidation with ceric sulphate[19],
b) oxidation with $KMnO_4$[20,21],
c) iodometrically[18].

Which of the three methods to use depends on whether and how the assay mixture is coloured.

It is important to take as many samples for estimation as possible during the first two minutes of the H_2O_2 decomposition. The rate of the enzyme reaction frequently decreases in this period.

The iodometric determination of H_2O_2 can be used as a macro-assay, or as a micromethod with tissue slices or small amounts of enzyme, or even as a submicrotitration[22]. With the technique of submicrotitration described by *Linderström-Lang*[23] and other workers a few microlitres are sufficient. The principle is the same as for the macro-assay.

Principle

Samples are removed at timed intervals from the reaction mixture and mixed with acid to stop enzyme activity. H_2O_2 is determined in these samples.

The hydrogen peroxide not decomposed by the catalase oxidizes potassium iodide to iodine. The iodine is titrated with thiosulphate solution using starch solution as indicator.

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18) *K. G. Stern*, Hoppe-Seylers Z. physiol. Chem. *204*, 259 [1932].
19) *A. Lembke* and *H. Menninger*, Kieler Milchwirtsch. Forsch. Ber. *4*, 56 [1952].
20) *H. v. Euler* and *G. Blix*, Hoppe-Seylers Z. physiol. Chem. *105*, 83 [1919].
21) *H. v. Euler* and *K. Josephson*, Liebigs Ann. Chem. *452*, 158 [1927].
22) *H. Frahm*, Zbl. Bakteriol., Parasitenkunde, Infektionskrankh. Hyg., I. Abt., Orig. *152*, 253 [1947].
23) *K. Linderström-Lang* and *H. Kolter*, Hoppe-Seylers Z. physiol. Chem. *201*, 9 [1931].

Optimum Conditions for Measurements

The details on p. 886 apply here also. The measurements can be carried out at much lower temperature with this method.

Reagents

1. Potassium dihydrogen phosphate, A. R., KH_2PO_4
2. Disodium hydrogen phosphate, A. R., $Na_2HPO_4 \cdot 2\ H_2O$
3. Hydrogen peroxide*), *ca.* 30% (w/v)
4. Sulphuric acid, conc.
5. Trichloroacetic acid
6. Potassium iodide, A. R.
7. Sodium thiosulphate, A. R., $Na_2S_2O_3 \cdot 5\ H_2O$
8. Sodium carbonate, $Na_2CO_3 \cdot 10\ H_2O$
9. Soluble starch
10. Mercuric iodide, HgI_2

Preparations of Solutions

I. Phosphate buffer (M/15; pH 7.0):
See spectrophotometric method (p. 886), solution I.

II. Hydrogen peroxide (*ca.* 2% w/v):
Dilute 7 ml. H_2O_2 (*ca.* 30% w/v) to 1000 ml. with distilled water.

III. Sulphuric acid-trichloroacetic acid:
Dissolve 40 g. trichloroacetic acid in distilled water and make up to 100 ml. To this solution carefully add 25 ml. concentrated H_2SO_4 in small portions; cool and mix.

IV. Potassium iodide (10% w/v):
Dissolve 10 g. KI in distilled water and make up to 100 ml.

V. Sodium thiosulphate (0.1 N):
Dissolve 24.82 g. $Na_2S_2O_3 \cdot 5\ H_2O$ and 0.1 g. $Na_2CO_3 \cdot 10\ H_2O$ in distilled water and make up to 1000 ml.

VI. Indicator (starch):
Stir 1 g. soluble starch and 5 mg. HgI_2 with water to form a paste, add to 500 ml. boiling water, allow to boil for a few minutes and then cool.

Stability of the solutions

The phosphate buffer and the starch solution are stable indefinitely if bacterial contamination is avoided. Likewise solution (III) keeps indefinitely. The hydrogen peroxide and KI solutions should be prepared freshly each day and the thiosulphate solution should be checked each week.

Procedure

Experimental material

Tissue: Extraction from plant and animal tissue, see p. 887. The activity of tissue slices can be obtained approximately if the assay mixture containing the slices is shaken during the enzymatic reaction.

Foodstuff: Milk can be used without preliminary treatment.

*) *e.g.* "Perhydrol" Merck.

With samples containing very little catalase the volumes of H_2O_2, buffer and sample should be altered, so that the measured values are sufficiently large. Or alternatively a more dilute thiosulphate solution can be used for the titrations. For example, milk is assayed without buffer solution, 30% H_2O_2 (not solution II) is added (0.27 ml./100 ml. milk) and it is titrated with 0.05 N $Na_2S_2O_3$ solution. The temperature of the measurements should preferably be between 0 and 10°C (refrigerated bath). A cold room can also be used. Equilibrate all solutions before starting the measurements.

Pipette into a 200 ml. Erlenmeyer flask:

50 ml. H_2O_2 solution (II)
40 ml. buffer (solution I).

Quickly blow in from a 10 ml. delivery pipette with a wide tip

10 ml. sample solution*),

shake and start a stopwatch.

Remove 10 ml. samples of the reaction mixture with a 10 ml. pipette (as above) at exactly 0.5, 1, 2, 4, 7 and 10 min. and blow into prepared centrifuge tubes. These contain:

2 ml. acid (solution III), with samples essentially free from protein *ca.* 7 N H_2SO_4 is sufficient.

After blowing in the

10 ml. reaction mixture,

quickly centrifuge off the precipitated protein**). Pipette 6 ml. of the supernatant into a 50 ml. ground-glass flask and add

2 ml. KI solution (IV).

Allow to stand for 20 min. stoppered, in the dark and then quickly titrate to a faint yellow colour with

thiosulphate solution (V)

from a 10 ml. burette. Add

2 drops of starch solution (VI)

and continue the titration until the blue colour disappears. Record the ml. of thiosulphate solution required.

Calculations

Since the quantity of the thiosulphate solution required is proportional to the amount of H_2O_2 left in the enzymatic reaction mixture (1 ml. 0.05 N thiosulphate solution $\triangleq$ 0.025 mmoles H_2O_2) the titration value in ml. can be inserted in equation (3), p. 886, without further conversion. Δt is the interval between the first and the second (or the third or fourth, *etc.*) sample. Calculate the k values for these intervals. If these are not constant (allowing for a reasonable scatter), but decrease with time, it is necessary to extrapolate to time $t = 0$. If k shows a significant dependence on the hydrogen peroxide concentration, then also extrapolate to $[H_2O_2] = 0$. Using the k value calculate the catalase units in the enzymatic reaction mixture according to equation (4), p. 888, or the "Katalase-fähigkeit" (Kat. f.) of the sample according to equation (6a), p. 893.

*) If less is available make up with buffer.
**) Can be omitted if there is only a slight turbidity due to the low protein content of the sample.

Example

Measurements on milk. Samples were removed at 30, 60, 150 and 300 sec. and required the following volumes of 0.05 N thiosulphate solution to titrate the residual peroxide: 4.37, 4.33, 4.23 and 4.08 ml. From the values for 30 and 60 sec., 30 and 150 sec. and 30 and 300 sec. the following values for k were obtained according to equation (3): 0.00031, 0.00027 and 0.00025 $sec.^{-1}$. Extrapolation to $t = 0$ gave $k = 0.00034\ sec.^{-1}$.

As the dry weight of the milk was 12.5% according to equation (6a), p. 893, this value for k corresponds to a "Katalasefähigkeit" (Kat. f.) of 1.4×10^{-3}.

C. Determination with the "Katalaser"[24-26]

Principle

"Katalaser" are graduated glass vessels for the volumetric measurement of the oxygen liberated enzymatically from hydrogen peroxide. The sample and the H_2O_2 solution are mixed in the "Katalaser" usually without the addition of buffer. After a fixed interval the volume of oxygen is read off.

Reagents

I. Phosphate buffer (M/150; pH 7):
Dilute solution I on p. 886 1:10 with distilled water.

II. Hydrogen peroxide:
30% (w/v) or 2% (w/v) or 1% (w/v).

Procedure

For the determination of catalase in milk according to[27]: Pipette into a "Katalaser":

10 ml. milk
2 ml. H_2O_2 solution (2% w/v).

Mix, allow to stand for 2 hours at room temperature and then read off the volume of oxygen.

For the determination of catalase in milk according to[28]: Pipette into a "Katalaser":

15 ml. milk
5 ml. H_2O_2 solution (1% w/v).

Mix, allow to stand for 2 hours at room temperature and read off the volume of oxygen.

Determination in powders, for example, flour, according to[29]: Stir 1 g. of flour with 100 ml. M/150 phosphate buffer, pipette

100 ml. of this suspension and
3.3 ml. H_2O_2 solution (30% w/v)

into a "Katalaser", allow to stand for 30 min. at room temperature and read off the volume of oxygen.

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24) *O. Lobeck*, Milchwirtsch. Zbl. *6*, 316 [1910].
25) *G. Roeder*, Molkerei-Ztg. *42*, 1591 [1928].
26) *G. Roeder*, Milchwirtsch. Forsch. *9*, 516 [1930].
27) *G. Roeder:* Grundzüge der Milchwirtschaft und des Molkereiwesens. Parey-Verlag, Hamburg 1954, p. 762.
28) *G. Schwarz, B. Hagemann, C. Hüttig, R. Kellermann* and *W. Staege:* Die chemischen und bakteriologischen Untersuchungsverfahren für Milch, Milcherzeugnisse und Molkereihilfsstoffe. Neumann-Verlag, Berlin 1950, p. 118.
29) *H. Thaler* in *E. Bamann* and *K. Myrbäck:* Die Methoden der Fermentforschung. G. Thieme, Leipzig 1941, Vol. III, p. 2844.

Calculations

Relate the ml. of oxygen to 100 ml. milk or to 1 g. of the powder (flour). According to *Koning*[30] the catalase number is the amount of H_2O_2 decomposed, measured in ml. oxygen, by 100 ml. milk in 2 hours. For flour it is the ml. O_2 liberated from 1 g. flour in 30 min.

Example

10 ml. of fresh milk, assayed 3 hours after milking, produced 2 ml. O_2 in 2 hours in the assay according to[27]. That is 20 ml./100 ml. milk. The catalase number is 20.

Definitions of Activity and Conversion Factors

The purity of a catalase preparation is defined by the quotient of the optical densities at 410 and 275 mμ. The optical density at 410 mμ (Soret band) represents the haem part of the molecule, while that at 275 mμ represents the protein content. The purity gives no indication of the enzymatic activity.

The activity is defined by the enzymatic action:

1. Catalase units

According to[13] a catalase unit is the amount of enzyme which liberates half the peroxide oxygen from a H_2O_2 solution in 100 sec. at 25°C. Therefore the following relationship exists (see p. 888) between activity and the rate constant (in sec.$^{-1}$):

$$\text{(5)} \qquad \text{units according to}^{12} = \frac{k}{6.93 \times 10^{-3}}$$

The International Unit[31] is defined as the μmoles of substrate converted per mg. protein per min. at 25°C. To convert to International Units the H_2O_2 concentration must be defined. It should be *ca.* 1.25×10^{-2} M (see p. 887).

If an optical density decrease from 0.450 to 0.400 is measured at 240 mμ according to p. 887, this means (according to the data of[6] ε for H_2O_2 is about 0.036 cm.^{2}/μmole) that in a 3 ml. assay mixture the initial H_2O_2 concentration is about 1.25×10^{-2} M = 37.5 μmoles H_2O_2/3 ml. and that about 4.2 μmoles of H_2O_2 are decomposed.

Since 1 unit according to[13] is defined for $\tau = 100$ sec. and therefore for a first order reaction $k = 6.93 \times 10^{-3}$ sec.$^{-1}$ (see p. 888), it follows that substituting this k value and $s_1 = 37.5$ μmoles H_2O_2/3 ml. in equation (3) gives a rate of decomposition of *ca.* 13 μmoles H_2O_2/min. Linear conversion of the value for 100 sec. to 60 sec. is not possible. Consequently: 13 International Units are equal to 1 unit according to[13].

$$\text{(5a)} \qquad 1 \text{ International Unit} = 13 \times \text{units according to}^{13}.$$

The conversion factor therefore depends on the H_2O_2 concentration used for the assay.

2. Katalasefähigkeit

The "Katalasefähigkeit" (Kat. f.) is defined*) as

$$\text{(6)} \qquad \text{Kat. f.} = \frac{K}{\text{g. sample (dry weight) in 50 ml. reaction mixture}}$$

where $K = \frac{1}{\Delta t} \log \frac{S_1}{S_2}$ [min.$^{-1}$].

*) The definition of Kat. f.[20,21,32] is based on a first order reaction rate constant. Although $k = \frac{1}{\Delta t} \ln \frac{S_1}{S_2}$ (refer to[33]), for the determination of Kat. f. $K = \frac{1}{\Delta t} \log \frac{S_1}{S_2}$ is used (see[32,34,35]).

30) *C. J. Koning*, Milchwirtsch. Zbl. *3*, 67 [1907].
31) *J. Cooper, P. A. Srere, H. Tabachnik* and *E. Racker*, Arch. Biochem. Biophysics *74*, 306 [1958].
32) *H. v. Euler* and *K. Josephson*, Chem. Ber. *59*, 770 [1926].
33) *H. v. Euler:* Chemie der Enzyme. F. J. Bergmann, Munich. 1934, part II, 3, p. 74.
34) *J. W. Sizer*, J. biol. Chemistry *154*, 461 [1944].
35) *R. K. Bonnichsen, B. Chance* and *H. Theorell*, Acta chem. scand. *1*, 685 [1947].

The Kat. f. can be calculated from the rate constant k (see equation (3), p. 886).

(6a) $$\text{Kat. f.} = \frac{60 \times k \times 3}{2.3 \times 10^{-3} \times 50 \times \text{mg. dry weight}} = 1565\ \text{k/mg. dry weight.}$$

The factors 60 and 2.3 convert from min. to sec. and from ln to log respectively, 10^{-3} from g. to mg. and 3/50 from 50 to 3 ml.; k can be expressed in catalase units according to[13]; according to equation (5)

$$k = \text{units} \times 6.93 \times 10^{-3},$$

substitution in equation (6a) gives

(6b) $$\text{Kat. f.} = 1565 \times 6.93 \times 10^{-3} \times \text{units/mg.}$$
$$= 10.8 \times \text{units according}^{13}\text{/mg.}$$

or according to equation (5a)

(6c) $$\text{Kat. f.} = 0.83 \times \text{International Units/mg.}$$

If the catalase activity (expressed as Kat. f.) of individual cells (bacteria, erythrocytes) is required, then divide k by the number of cells in the reaction mixture instead of by the dry weight of the sample. This gives the "Katalase-Wirksamkeit"[36] or catalase index[37].

Conversion Table*)

To convert from	to	multiply by
Kat. f.	Units[13]/mg.	0.093
	International Units/mg.	1.2
Units[13]/mg.	Kat. f.	10.8
	International Units/mg.	13
International Units/mg.	Kat. f.	0.83
	Units[13]/mg.	0.077

3. The catalase number

This definition of activity is very common in food chemistry. For the definition, see p.717. The volume of O_2 formed or the residual amount of H_2O_2 is measured volumetrically or by titration after a relatively long incubation period. Since the amount of catalase in biological material is often small and since H_2O_2 destroys the enzyme, the reaction frequently stops before all the peroxide is decomposed. The amount of H_2O_2 hydrolysed before the complete inactivation of the catalase is approximately proportional to the enzyme concentration, but it also depends on the temperature of the reaction and on the inactivation constant of the catalase[38]. The latter varies greatly with different catalases. The inactivation time for the amounts of catalase usually used is about 2 hours. The measurement of the catalase number is in reality the estimation of the maximum amount of H_2O_2 decomposed, although this fact is not usually emphasized.

It is impossible to calculate the rate constant k from the catalase number in order to convert to other units.

*) The dependence of the reaction on temperature has not been taken into account in the conversion of the Kat. f. values measured at 0°C into the units measured at 25°C.

36) *A. I. Virtanen* and *H. Karström*, Biochem. Z. *161*, 10 [1925].
37) *G. J. von Thiemen*, Zbl. Biochem. Biophys. *20*, 159 [1919].
38) *H. Lück*, Biochem. Z. *329*, 165 [1957].

4. The catalase concentration

The rate constant k of the catalase reaction includes the enzyme concentration e:

(7) $$k = e \times k'$$

Knowing k′ (of the order of 10^7 $mole^{-1} \times sec.^{-1}$) it is possible to calculate e:

(7a) $$e = \frac{k}{k'} \text{ [mole/l.]}$$

Several k′ values are given in the following Table:

Crystalline catalase from	k′ [$mole^{-1} \times sec.^{-1}$]
Erythrocytes (human)[39]	3.4×10^7
Erythrocytes (horse)[35]	3.5×10^7
Erythrocytes (horse)[40]	6.6×10^7
Liver (bovine)[39]	2.9×10^7
Liver (horse)[35]	3.0×10^7
Bacteria[41]	5.3×10^7

[39] *B. Chance* and *A. C. Maehly* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1955, Vol. II, p. 765.
[40] *H. F. Deutsch*, Acta chem. scand. *5*, 815 [1951].
[41] *B. Chance* and *D. Herbert*, Biochem. J. *46*, 402 [1950].