Distribution. Besides being present in *Pseudomonas fluorescens*, appreciable cytochrome peroxidase activity was found in *Azotobacter vinelandii*, and the *Poky* mutant of *Neurospora crassa*. Assays of extracts of *E. coli*, BCG, *Clostridium kluyveri*, *Neurospora crassa*, and *Azotobacter agile* demonstrated the presence of small amounts of cytochrome peroxidase activity only in the presence of 10^{-3} *M* sodium azide. The azide was added in order to inhibit the catalase; it had no significant effect on the peroxidase. Apparently, the ratio of catalase to cytochrome peroxidase was very high in these organisms, and the hydrogen peroxide was available to the peroxidase when the catalase was inhibited.

Extracts of soybean leaves exhibited a very high specific activity. However, it cannot be definitely said whether the activity of the soybean extract was due to the cytochrome peroxidase, because it has not been possible to observe the cytochrome pigment in these extracts. The activity may be due to another peroxidase mediating the oxidation of the dye. In any case the activity exhibited by the soybean extract is due solely to a peroxidase, as it is completely inhibited by catalase.

[136] Assay of Catalases and Peroxidases

$$\text{Catalase:} \qquad 2H_2O_2 \rightarrow 2H_2O + O_2 \qquad (1)$$

$$\text{Catalase and Peroxidase:} \quad ROOH + AH_2 \rightarrow H_2O + ROH + A \qquad (2)$$

By BRITTON CHANCE and A. C. MAEHLY

I. Catalase Assay by Disappearance of Peroxide

A. Ultraviolet Spectrophotometry

Principle. On the basis of the absorption curves for peroxide solutions (Lederle and Rieche[1]), Chance and Herbert[2] devised a method for determining the activity of catalase by direct measurements of the decrease of light absorption in the region 230 to 250 mμ caused by the decomposition of hydrogen peroxide by catalase. More detailed procedures have been published.[3,4]

Range of Usefulness. This method is limited to the assay of catalase solutions that are pure enough to give negligible absorption at 230 to

[1] E. Lederle and A. Rieche, *Ber.* **62,** 2573 (1929).
[2] B. Chance and D. Herbert, *Biochem. J.* **46,** 402 (1950).
[3] R. F. Beers, Jr., and I. W. Sizer, *J. Biol. Chem.* **195,** 133 (1952).
[4] B. Chance, *in* "Methods of Biochemical Analysis" (Glick, ed.), p. 408, Interscience Publishers, New York, 1954.

250 mμ in the concentrations required for the assay. Assays according to equation 2 have not been studied in detail but are apparently possible.[5]

Reagents

0.01 *M* phosphate buffer, pH 7.0.
Stock solution of H_2O_2 (~1 *M*).
Stock solution of catalase (~10^{-4} *M* or less).

Spectrophotometric Determination of Catalase Concentration. The molecular extinction coefficients of the useful absorption bands of various types of catalases are presented in Table I and can be used for measuring

TABLE I

MOLECULAR WEIGHTS, EXTINCTION COEFFICIENTS, AND k_1' VALUES (EQUATION 8) FOR CATALASES FROM VARIOUS SOURCES

Catalase source	Purified by	Molecular weight	ϵ_{405}, $mM^{-1} \times cm.^{-1}$	k_1', $10^7 \times M^{-1} \times sec.^{-1}$
Human liver	Bonnichsen[a]	—	290[a,b]	—
Horse liver	Dounce and Frampton[c]	225,000[d]	340[d]	3.0[e]
Beef liver	Sumner and Dounce[f]	225,000[g]	—	2.9[h]
		251,000[i]		
Human erythrocytes	Herbert and Pinsent[j]	220,000[j]	—	3.4[h]
Horse erythrocytes	Bonnichsen[k]	(225,000[k])	378[k]	3.5[e]
		250,000[l]	402[l]	6.6[m]
Human kidney	Bonnichsen[a]	—	366[a,b]	—
Bacteria	Herbert and Pinsent[n]	232,000[n]	410[o]	5.3[o]

[a] R. K. Bonnichsen, *Acta Chem. Scand.* **1,** 114 (1947).
[b] These values are approximate since the molecular weight is estimated.
[c] A. L. Dounce and O. D. Frampton, *Science* **89,** 300 (1939).
[d] K. Agner, *Biochem. J.* **32,** 1702 (1938).
[e] R. K. Bonnichsen, B. Chance, and H. Theorell, *Acta Chem. Scand.* **1,** 685 (1947).
[f] J. B. Sumner and A. L. Dounce, *Science* **85,** 366 (1937).
[g] J. B. Sumner, A. L. Dounce, and O. D. Frampton, *J. Biol. Chem.* **136,** 343 (1940).
[h] A. C. Maehly, *in* "Methods in Biochemical Analysis" (Glick, ed.), p. 358, Interscience Publishers, New York, 1954. Calculated from published *Kat.f.* values.
[i] M. Shirakawa, *J. Fac. Agr. Kyushu Univ.* **9,** 173 (1949).
[j] D. Herbert and J. Pinsent, *Biochem. J.* **43,** 203 (1948).
[k] R. K. Bonnichsen, *Arch. Biochem.* **12,** 83 (1947).
[l] H. F. Deutsch, *Acta Chem. Scand.* **6,** 1516 (1952).
[m] H. F. Deutsch, *Acta Chem. Scand.* **5,** 815 (1951).
[n] D. Herbert and J. Pinsent, *Biochem. J.* **43,** 193 (1948).
[o] B. Chance and D. Herbert, *Biochem. J.* **46,** 402 (1950).

[5] B. Chance, *in* "Investigation of Rates and Mechanisms of Reactions" (Friess and Weissberger, eds.), p. 637, Interscience Publishers, New York, 1953.

the catalase concentration as is required for the computation of the specific activity as described below. Alternatively, the dry weight of enzyme used in the assay is divided by the molecular weight.

Apparatus. Matched cuvettes, of 1-cm. optical path and 3-ml. volume (the cuvettes should be clean enough so that the "blank" rate of peroxide decomposition is negligible); an ultraviolet spectrophotometer (Beckman, Unicam, Uvispek, etc.) having sufficient electrical stability so that the "drift" of the optical density reading is less than 0.005 per minute; a micropipet delivering about 1 μl. accurate to 2%.

Procedure. At temperatures of 26° and less where convenient, the optical density of a 1:500 H_2O_2 solution is measured in the region 230 to 250 mμ (this is x_0). The apparatus is left set for the optical density reading (Beckman selector switch set at "1"), and the shutter is closed. About 1 μl. of catalase is placed as a drop on the end of a stirring rod, is rapidly stirred into the peroxide solution, and a stop watch is started. The cover for the cuvette holder is rapidly replaced, the shutter opened, and "tracking" begun (the null galvanometer of the spectrophotometer is kept continuously zeroed by a slow, continuous rotation of the optical density knob). The stop watch and the slowly changing optical density value are read every 10 seconds for the first 30 seconds to give the quantity x. Thereafter the optical density of the solution is measured in the usual manner by taking readings on both cuvettes at 50 and 70 seconds. Thus five readings of the quantity x are obtained. The catalase concentration is adjusted so that about half the peroxide is decomposed in about 30 seconds. If both the catalase and the spent hydrogen peroxide solutions absorb negligibly at 230 to 250 mμ, as is usually the case, the end point of the reaction will correspond closely to zero optical density. If impure catalase is used or if the spent hydrogen peroxide solution contains absorbing substances, the end point of the reaction should be measured and subtracted from the optical densities read in the course of the reaction. If this proves inconvenient, an alternative form of calculation is provided below.

Calculation of Results. Under these experimental conditions the kinetics of hydrogen peroxide disappearance closely follow first-order kinetics and the velocity constant (k) is calculated:

$$k = \frac{2.3}{t} \log \frac{x_0}{x} \tag{3}$$

The units are sec.$^{-1}$. A fairly constant value of k is obtained with most catalases from the five determinations. If the catalase is extremely labile, the value of k may be taken by extrapolation of the experimental values to $t = 0$.

An alternative form of equation 3 that is sometimes useful is

$$k = \frac{2.3}{t_2 - t_1} \log \frac{x_1}{x_2} \tag{4}$$

where t_1 and t_2 are the times corresponding to a pair of readings of the optical densities x_1 and x_2.

The quantity k is used in two ways:

1. *To calculate the specific activity of catalase* (k_1'). The quantity k_1' is calculated from k by dividing the latter by the enzyme molarity, e, as determined spectrophotometrically.

$$k_1' = k/e\ M^{-1} \times \text{sec.}^{-1} \tag{5}$$

The quantity k_1' is related to the two principal steps of catalase action,[6]

$$E + S \xrightarrow{k_1} ES \tag{6}$$

$$ES + S \xrightarrow{k_4'} E + P \tag{7}$$

by the equation

$$k_1' = \frac{1}{\frac{1}{k_1} + \frac{1}{k_4'}} \tag{8}$$

Representative values of k_1' for various catalases are given in Table I. This method should be used to define the specific activity of a new catalase. If the value obtained for a known catalase is less than that given in Table I, the catalase (1) contains impurities that absorb light at the wavelengths used to determine the enzyme concentration spectrophotometrically, (2) contains a larger proportion of inactive hematin groups in the case of liver catalases, (3) represents a less active fraction of erythrocyte catalases.

2. *To calculate the value of Kat.f.* This quantity, although it involves a confusion of units, is still used for the comparison of catalase activity and purity.

$$Kat.f. = 60k/2.3W \tag{9}$$

Here k is converted from sec.$^{-1}$ to min.$^{-1}$ by multiplying by 60. Naperian logarithms are converted back to Briggsian by the factor 2.3. The term W is the grams of catalase in a final reaction mixture of 50-ml. volume and is $5\frac{0}{3}$ times the weight of catalase actually used in the method described here.

Kat.f. is not suitable for comparing the activity of catalases of different molecular weights, and the specific activity k_1' should be used.

[6] B. Chance, D. S. Greenstein, and F. J. W. Roughton, *Arch. Biochem. and Biophys.* **37**, 301 (1952).

Accuracy of the Method. The results should be accurate to a few per cent and should agree to within that accuracy with the results obtained by rapid titration methods (see below). For bacterial catalase, Chance and Herbert[2] obtained $k_1' = 5.35$ and 5.3×10^7 at 25° for rapid titrimetric and ultraviolet spectrophotometric assays of bacterial catalase.

B. Permanganate Titration

For those who have no ultraviolet spectrophotometer available or who wish to assay a very impure catalase, we include a brief summary of the titrimetric method.[7–9]

Reagents

0.25 *N* hydrogen peroxide.
0.01 *M* phosphate buffer, pH 7.0.
Stock catalase solution diluted to about 1 μM
0.01 *N* potassium permanganate.
2% sulfuric acid solution.

At a temperature of 20° or less when convenient, 15 ml. of 0.01 *N* hydrogen peroxide (1:25 in phosphate buffer) is placed in an Erlenmeyer flask, and 2.0 ml. of this solution is withdrawn and added to an excess of 2% sulfuric acid for titration with permanganate with an accuracy of 1.0%. Thus the quantity x_0 is determined. Then 0.030 ml. of the catalase is pipetted onto a watch glass and is dropped into the peroxide solution which is being swirled rapidly. The stop watch is started at the same time. Samples of 2 ml. each are withdrawn at various times and are rapidly blown out (through a wide-tipped pipet) into separate flasks containing excess 2% sulfuric acid that is rapidly swirling at the time the sample is blown out. It is possible to withdraw samples and blow them into the acid at 10, 20, 30, and 50 seconds after adding catalase. Permanganate titration of these solutions gives the values of x. The methods of calculation are the same as those described above.

II. Method for Crude Cell Extracts

Although it is now generally recognized that current manometric methods for catalase assay are completely unsuitable for accurate determination of catalase activity,[9,10] relative values of activity can be determined under conditions where other methods are inapplicable.

[7] H. von Euler and K. Josephson, *Ann.* **455,** 1 (1927).
[8] R. K. Bonnichsen, B. Chance, and H. Theorell, *Acta Chem. Scand.* **1,** 685 (1947).
[9] A. C. Maehly, *in* "Methods of Biochemical Analysis" (Glick, ed.), p. 358, Interscience Publishers, New York, 1954.
[10] K. Agner and H. Theorell, *Arch. Biochem.* **10,** 321 (1946).

Principle. Oxygen evolution caused by the decomposition of hydrogen peroxide is measured with the conventional manometric technique.

Range of Usefulness. This is a method of last resort but may be used (1) when purification of the enzyme is difficult and the losses of such a procedure are not controlled; (2) when the cell extracts are too turbid to permit the use of the ultraviolet spectrophotometric method; (3) when the material contains substances that react with permanganate or interfere with polarographic methods.[4]

Procedure. When a conventional manometric technique is used, the main compartment of the vessel should contain 3 ml. of 0.01 *M* phosphate buffer, pH 7.0, and 0.2 ml. of 0.2 *M* hydrogen peroxide. The catalase may be placed in the side arm but is preferably contained in a "dangling cup"[11] or a magnetically released vessel[12] (Will Corporation, New York 12, N.Y.). Readings should be made at 1, 2, and 3 minutes after adding catalase. The temperature can be 20° or below, and the shaking rate 100 to 200 strokes per minutes.

Calculation of Results. Since this method is recommended only for relative assays and not for the calculation of k_1' or *Kat.f.*, the results may best be expressed in values relative to those of some standard material such as liver, where the catalase content is known on the basis of purification procedures.

III. Direct Spectrophotometric Assay of Catalase and Peroxidase in Cells and Tissues

In biological materials that contain relatively large amounts of catalase, Chance[13] has shown that catalase may be assayed directly in the cells by means of sensitive spectrophotometric methods for the detection of changes of absorption that occur when an enzyme-substrate compound of catalase is formed or is decomposed. This method may ultimately replace the manometric one in many cases. It has also been possible to measure the formation of an enzyme-substrate compound of peroxidase in yeast cells by the addition of methyl hydrogen peroxide.[13]

IV. Peroxidase Assay by Spectrophotometric Measurements of the Disappearance of Hydrogen Donor or the Appearance of Their Colored Oxidation Products

General Principles. Peroxidases catalyze the oxidation by peroxide of a wide variety of substances many of which have strong absorption bands

[11] D. Keilin and E. F. Hartree, *Proc. Roy. Soc.* (*London*) **B117,** 1 (1935).
[12] Lord Rothschild, *J. Exptl. Biol.* **26,** 396 (1950).
[13] B. Chance, *in* "The Mechanism of Enzyme Action" (McElroy and Glass, eds.), p. 399, The Johns Hopkins Press, Baltimore. 1954.

themselves or the oxidation products of which absorb strongly.[9] But in the peroxidases it is necessary to distinguish the two types of specific activity that may be measured, depending on the experimental conditions: (1) the velocity constant for the formation of the enzyme-substrate complex:

$$E + S \xrightarrow{k_1} ES_{\mathrm{I}} \tag{10}$$

and (2) the velocity constant for the reaction of the secondary complex with the hydrogen donor molecule:

$$ES_{\mathrm{II}} + AH \xrightarrow{k_4'} E + P \tag{11}$$

We shall describe methods that approximate the measurement of each of these quantities or point out where current assay methods fail to measure either one. The use of guaiacol makes it possible to approximate k_1 or k_4; pyrogallol gives no satisfactory approximation to k_4; the mesidine test (Table III) has not been proved to measure k_1 or k_4. With the other peroxidases, lacto-, verdo-, and cytochrome c or yeast peroxidase, the assay conditions are not nearly so well worked out and the assay procedures may give only a mixture of the specific activities of the two rate-determining steps in peroxidase action.

A. The Guaiacol Test

Principle.[14] The rate of utilization of peroxide (dx/dt) to form the colored reaction product,[15] as measured at 470 mμ, depends on the respective concentrations of substrate (peroxide) and donor (guaiacol) (equations 10 and 11) in the following way:

$$\frac{dx}{dt} = \frac{e}{\dfrac{1}{k_4 a_0} + \dfrac{1}{k_1 x_0}} \tag{12}$$

where e = concentration of enzyme.
a_0 = initial concentration of donor.
x_0 = initial concentration of substrate.

If the conditions of the assay are chosen so that $k_4 a_0 \gg k_1 x_0$, equation 10 represents the rate-limiting step and we obtain k_1:

[14] This method was developed by Mr. T. M. Devlin of this laboratory for the assay of cytochrome c peroxidase, and Devlin's method was used by Dr. P. George in 1953 during his visit here [see P. George, *J. Biol. Chem.* **201**, 413 (1953)].

[15] A formulation of this reaction can be found elsewhere.[9]

$$\frac{dx}{dt} \approx k_1 x_0 e \tag{13a}$$

and

$$k_1 \approx \frac{1}{x_0 e} \times \frac{dx}{dt} \approx \frac{1}{x_0 e} \times \frac{\Delta x}{\Delta t} \tag{13b}$$

where $\Delta x/\Delta t$ is the rate of substrate disappearance over the measured time interval.

However, if the assay is adjusted so that $k_1 x_0 \gg k_4 a_0$, k_4 can be obtained, since in this case

$$\frac{dx}{dt} \approx k_4 a_0 e \tag{14a}$$

and

$$k_4 \approx \frac{1}{a_0 e} \times \frac{\Delta x}{\Delta t} \tag{14b}$$

The experimental procedure is the same for both modifications of the test.

Reagents

20 mM guaiacol (0.22 ml. of guaiacol in 100 ml. of water).
10 mM phosphate buffer, pH 7.0.
10 mM hydrogen peroxide solution (for k_1 determination).
40 mM hydrogen peroxide solution (for k_4 determination).
$\sim 10^{-7}$ M peroxidase solution.

Procedure. The values b, c, d, and f depend on the constant to be measured and are read from Table II.

TABLE II

AMOUNTS OF REAGENTS ADDED TO THE CUVETTE AND THE FINAL CONCENTRATION OF THESE REAGENTS FOR THE TWO MODIFICATIONS OF THE GUAIACOL ASSAY

	Units	Symbol used in text	Constant to be determined	
			k_1	k_4
Buffer	ml.	b	1.0	2.9
Guaiacol	ml.	c	2.0	0.05
[Guaiacol]	M	a_0	1.3×10^{-2}	3.3×10^{-4}
H_2O_2 stock	mM	d	10	40
[H_2O_2]	M	x_0	3.3×10^{-5}	1.3×10^{-4}
D_f[a]	cm.$^{-1}$	f	0.2	0.8
$k_1 x_0$	—	—	300	1170
$k_4 a_0$	—	—	4300	110

[a] Approximate final reading of optical density at 470 mμ.

A spectrophotometer or direct-reading colorimeter suitable for a wavelength region of 470 mμ may be used. The assay is carried out at room temperature. One of a pair of cuvettes of 1-cm. path is filled with water, the other with b ml. of phosphate buffer, c ml. of guaiacol, and about 10^{-9} M peroxidase (see below). The initial optical density is read at 470 mμ, and the optical density scale is offset to a value 0.050 greater than this reading. The shutter is closed, and 10 μl. of d mM hydrogen peroxide is added as a drop at the end of a stirring rod (the final concentration is x_0). The solution is rapidly stirred, and a stop watch is started. The shutter is opened, and the time required (Δt) for the galvanometer to reach the null point is measured; it should be between 15 and 30 seconds. The end point of the reaction should correspond to an optical density of f.

Determination of the Enzyme Concentration. The peroxidase concentration can be determined spectrophotometrically on the basis of the molecular extinction coefficients given on pp. 799, 812, 817. Alternatively, the dry weight of the enzyme used may be divided by the molecular weight of the peroxidase (pp. 798, 808, 817) to give an effective concentration for an impure enzyme solution.

Calculation of the Results. The hydrogen peroxide utilized in the formation of a tetraguaiacol solution that gives an optical density of 0.05 at 470 mμ is $\frac{4 \times 0.050}{26.6}$ mM = 7.5 μM, since 4 moles of H_2O_2 is required to form 1 mole of tetraguaiacol and since the latter has an extinction coefficient of ϵ = 26.6 cm.$^{-1}$ mM^{-1} at 470 mμ.[9] This 7.5 μM of H_2O_2 corresponds to Δx of equations 13b and 14b, and Δt (units sec.) to the measured time interval. Both x_0 and e (units M. $\times$ l.$^{-1}$) can be measured spectrophotometrically (see above).

From equations 13b and 14b the rate constants k_1 and k_4, respectively, can thus be calculated:

$$k_1 = \frac{1}{x_0 e} \times \frac{\Delta x}{\Delta t} = \frac{7.5 \times 10^{-6}}{3.3 \times 10^{-5}} \times \frac{1}{e\,\Delta t} = \frac{.22}{e\,\Delta t} \qquad (15)$$

Experiments with pure horseradish peroxidase gave under the conditions of the test $k_1 = 0.89 \times 10^7$ at 20°, and 1.03×10^7 at 30°. The value of k_1 should be 0.9×10^7 at 25°.[16]

$$k_4 = \frac{1}{a_0 e} \times \frac{\Delta x}{\Delta t} = \frac{7.5 \times 10^{-6}}{3.3 \times 10^{-4}} \times \frac{1}{e\,\Delta t} = \frac{2.2 \times 10^{-2}}{e\,\Delta t} \qquad (16)$$

Actual experimental values were $k_4 = 2.2 \times 10^5$ at 20°, and 3.1×10^5 at 30°; k_4 should be 3.3×10^5 at 25°.[17]

[16] B. Chance, *Arch. Biochem.* **22,** 224 (1949).
[17] B. Chance, *Arch. Biochem.* **24,** 410 (1949).

The value of k_1 for lactoperoxidase, verdoperoxidase, and yeast peroxidase (cytochrome c peroxidase) is of the same order of magnitude as that for the horseradish enzyme, but experimental tests have not been made to determine whether this value can actually be obtained in the guaiacol test with any but the horseradish enzyme.

Accuracy and Limitations of the Method. The results should be accurate to a few per cent. The main sources of error are caused by the use of this method with crude cell extracts. In some cases there are substances present that interfere with the formation of tetraguaiacol.[17a]

B. The Pyrogallol Test

Principle. The traditional test for peroxidase activity is the formation of purpurogallin from pyrogallol (Willstätter and Stoll[18]). This method was devised before the mechanism of peroxidase action was fully understood. The original experimental conditions seem not to be suitable for measuring k_4 of equation 12, since $k_1x_0 \sim k_4a_0$, and it was demonstrated[17] that the enzymatic reaction proper was terminated (the enzyme-substrate compounds had disappeared) before all the end product (purpurogallin) had been formed. This shows that intermediate products must be involved and that the color formation is not really a direct measurement of enzyme kinetics. The assay will nevertheless be described for those who wish to correlate their data with those of the older literature.

Reagents. All solutions should be made up in glass-distilled water, and rigidly cleaned vessels should be used.

Pyrogallol (two times resublimed), 1.25 g. in 500 ml. (20 mM).

Hydrogen peroxide, 12.5 mg. in 500 ml. (0.74 mM).

Phosphate buffer, pH 7.0, 10 mM.

Peroxidase, about 1 γ in 500 ml. (5×10^{-11} M).

Procedure. In one of the modified procedures the reagents are made up in a 500-ml. volume, and the reaction is allowed to proceed for 5 minutes after which time it is stopped by adding 5 ml. of 5 N H_2SO_4. The purpurogallin is extracted three times with ether, alcohol is added, and the solution is made up to a known volume. The concentration of purpurogallin is determined spectrophotometrically at 430 mμ, where the extinction coefficient $\epsilon = 2.47$ cm.$^{-1}$ mM^{-1}.[19] A large number of varia-

[17a] It must be ascertained, therefore, that no secondary reactions with guaiacol or tetraguaiacol occur. Failure to take such reactions into account can give rise to misleading conclusions. Thus, P. George [*J. Biol. Chem.* **201,** 413 (1953)] observed that HOCl reacts with guaiacol or its oxidation products directly.

[18] R. Willstätter and A. Stoll, *Ann.* **416,** 21 (1917).

[19] A. C. Maehly, unpublished experiments.

tions of this procedure have been used, but as long as the concentration of the reactants is unchanged, the same result should be obtained.

Calculation of Results. The activity of peroxidase is traditionally expressed by the purpurogallin number (PZ). PZ is the number of milligrams of purpurogallin formed by 1 mg. of enzyme under the conditions of the standard test. The milligrams of enzyme present are determined directly by a dry weight determination or spectrophotometrically.[20] The value of PZ for pure peroxidase as determined by the test is 1020 according to Theorell and Maehly,[23] and 1220 according to Keilin and Hartree.[24] The value of PZ reached with equation 12 and the known values of k_1 and k_4 is 1500 (Chance[17]), which is somewhat in excess of that actually realized under the assay conditions.

TABLE III
MISCELLANEOUS PEROXIDASE TESTS

Peroxidase	Donor employed	Investigator	Cf. page
Horseradish	Mesidine	Paul and Avi-Dor[a]	
Yeast	Cytochrome c	Altschul *et al.*[b]	
Myelo-	Uric acid	Agner[c]	794
Lacto-	Dihydroxyphenylalanine	Polis and Shmukler[d]	813

[a] K. G. Paul and Y. Avi-Dor, *Acta Chem. Scand.* **8,** 649 (1954).
[b] A. M. Altschul, R. Abrams, and T. R. Hogness, *J. Biol. Chem.* **136,** 777 (1940).
[c] K. Agner, personal communication.
[d] B. D. Polis and H. W. Shmukler, *J. Biol. Chem.* **201,** 475 (1953)

Limitations of the Method. Considerable care is necessary to obtain reproducible results in the purpurogallin test (see Reagents). In addition, the test gives very low values for lacto- and verdoperoxidase, owing to the fact that these enzymes are not saturated with peroxide under the conditions of the test and are to some extent inactivated by the high H_2O_2 concentration. Thus their respective PZ values of 71 (Theorell and Åkeson[25]) and 41 (Agner[26]) cannot be converted into a specific rate constant. Direct measurements of k_4 from the kinetics of an enzyme-substrate compound of lactoperoxidase in the presence of pyrogallol gives a

[20] The molecular weight of horseradish peroxidase is 40,200 according to Theorell and Ehrenberg,[21] and 39,800 according to Cecil and Ogston.[22]
[21] A. Ehrenberg, personal communication; cf. A. C. Maehly, Vol. II [143], p. 808.
[22] R. Cecil and A. G. Ogston, *Biochem. J.* **49,** 105 (1951).
[23] H. Theorell and A. C. Maehly, *Acta Chem. Scand.* **4,** 422 (1950).
[24] D. Keilin and E. F. Hartree, *Biochem. J.* **49,** 88 (1951).
[25] H. Theorell and Å. Åkeson, *Arkiv Kemi, Mineral. Geol.* **17B,** No. 7 (1943)
[26] K. Agner, *Acta Physiol. Scand.* **2,** Suppl. 8 (1941).

value of 7×10^6, considerably greater than the value for the horseradish enzyme.[27]

C. Other Peroxidase Assays

Those working on the purification of peroxidases have usually developed their own particular assay system that should probably be followed by those who wish to duplicate the preparations. Table III lists some of these methods, which, however, do not clearly define a single reaction velocity constant. The guaiacol test is satisfactory for the enzymes listed in Table III, although it has not yet been proved that accurate values of k_1 and k_4 can be obtained in all cases.

[27] B. Chance, *J. Am. Chem. Soc.* **72,** 1577 (1950).

[137] Liver Catalase

By JAMES B. SUMNER and ALEXANDER L. DOUNCE

I. Introduction

Next to urease, beef liver catalase is possibly the easiest enzyme to obtain in crystalline condition. The reasons for this are the unusual stability of this enzyme, its insolubility in water at its isoelectric point, and its relatively high concentration in beef liver.

Since the preparation of crystalline catalase from beef liver by Sumner and Dounce[1] crystalline catalases have been obtained from a number of other sources. These are: lamb liver,[2] horse liver,[3] beef erythrocytes,[4] horse kidney and human liver,[5] guinea pig liver,[6] *Micrococcus lysodeikticus*,[7] and pig liver.[8]

Recently a method has been reported but not described for obtaining crystalline catalase from rat liver.[9]

Methods for preparing catalase from the livers of animals other than the ox are generally rather involved and for that reason will not be described here.

[1] J. B. Sumner and A. L. Dounce, *J. Biol. Chem.* **121,** 417 (1937).
[2] A. L. Dounce, *J. Biol. Chem.* **143,** 497 (1942).
[3] A. L. Dounce and O. D. Frampton, *Science* **89,** 300 (1939).
[4] M. Laskowski and J. B. Sumner, *Science* **94,** 615 (1941).
[5] R. K. Bonnichsen, *Acta Chem. Scand.* **1,** 114 (1947); *Arch. Biochem.* **12,** 83 (1947).
[6] R. K. Bonnichsen, *Acta Chem. Scand.* **2,** 561 (1948).
[7] D. Herbert and A. J. Pinsent, *Nature* **160,** 125 (1947).
[8] N. K. Sarkar and J. B. Sumner, *Enzymologia* **14,** 280 (1951).
[9] R. E. Greenfield and V. E. Price, *Proc. Am. Assoc. Cancer Research* **1,** 21 (1953).