In this mechanism equations (1) and (2) are chain-initiating, equations (3), (4) and (5), chain-carrying. The main support of the scheme is a good precedent for the real occurrence of reaction (4) in a model compound. An analogous reaction has been postulated by GEORGE[2] to explain the kinetics of the autooxidation of ferrous ion. I wish to point out that good evidence for the occurrence of the reaction (5) with divalent iron porphyrine complexes has been in the literature for some time, but it has been overlooked, possibly because of its very specialized character.

Very small amounts of hemine change characteristically the polarographic electroreduction of oxygen[3]. This change consists in the shift of a part of the wave due to the reduction of hydrogen peroxide to a more positive potential. The height of the new wave, which belongs to a catalyzed reduction of hydrogen peroxide, and its position on the current voltage curve, depends on the concentration of hemine and on the pH of the solution.

The half-wave potential of the catalyzed wave approaches the redox potential of hemine when the height of the catalyzed wave is small in comparison with the uncatalyzed one[4]. It has been shown that there are two interpretations which are in the first approximation polarographically equivalent.

One scheme may be represented by equations (6) and (7),

$$2\,Fe_h^{++} + H_2O_2 + 2H^+ \xrightarrow{k} 2\,Fe_h^{+++} + 2\,H_2O \qquad (6)$$

$$Fe^{+++} + e \longrightarrow Fe^{++} \qquad (7)$$

and it assumes a rapid oxidation of Fe_h^{++} by hydrogen peroxide followed by the reversible reduction of Fe_h^{+++} at the dropping mercury electrode.

The second scheme [equations (8) and (9)] assumes that Fe_h^{++} forms a 'complex' with hydrogen peroxide and this 'complex' is reducible on the dropping mercury electrode at a potential more positive than the redox potential of the Fe_h^{+++}/Fe_h^{++} system.

$$Fe_h^{++} + H_2O_2 \xrightarrow{k} Fe_h^{++}.(H_2O_2) \qquad (8)$$

$$Fe_h^{++}.(H_2O_2) + 2H^+ + 2e \longrightarrow Fe_h^{++} + 2H_2O \qquad (9)$$

Already at this stage, it was clear that the second explanation is the correct one as with many ferrohem complexes the oxidation with hydrogen peroxide does not proceed sufficiently rapidly to explain the polarographic effect.

The phenomenon was re-investigated recently by HANUŠ[5] in the light of the enormous progress which the Prague polarographic school has achieved in the analysis of kinetic and catalytic currents[6]. HANUŠ has shown that the second scheme is the correct one and that the formation of the 'complex' $Fe_h^{++}.(H_2O_2)$ is a reversible reaction. Consequently HANUŠ represented the mechanism of the catalyzed reduction of hydrogen peroxide in the presence of iron porphyrine complexes by the equations (10) and (11). Now it is not possible to calculate the rate constant k_1 for the reaction (10) from the polarographic measurements since this would require the knowledge of the value of the equilibrium constant for this reaction.

$$Fe_h^{++} + H_2O_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Fe_h^{++}.(H_2O_2) \qquad (10)$$

$$Fe_h^{++}.(H_2O_2) + 2H^+ + 2e \longrightarrow Fe_h^{++} + 2H_2O \qquad (11)$$

It is also impossible to obtain precisely analogous data with catalase itself[7]. However, one can estimate that the reaction (10) for ferrohem must be at least as fast as the decomposition of hydrogen peroxide by catalase, since the assumption of any finite value for k_1/k_2 [in equation (10)] must make k_1 larger than the value of k calculated for the case of an irreversible reaction.

The correspondence of the reaction (10) in the polarographic scheme with reaction (5) in the Westheimer mechanism is now obvious. We can clearly assume that either the complex detected by polarography should be written Fe_hO^{++} instead of $Fe_h^{++}.(H_2O_2)$ and that its observed formation is a model for Westheimer's reaction (5), or that the formation of a complex $P \cdot Fe^{++} \cdot (H_2O_2)$ analogous to the polarographic reaction should be inserted into Westheimer's scheme as the first stage of reaction (5), i.e.

$$P.Fe^{++} + H_2O_2 \rightleftharpoons P.Fe^{++}.(H_2O_2) \qquad (5a)$$

$$P.Fe^{++}.(H_2O_2) \longrightarrow P.FeO^{++} + H_2O \qquad (5b)$$

In both cases, the polarographic work cited seems to constitute a strong support of the equation (5) in Westheimer's scheme[8].

Zusammenfassung. Es werden polarographische Befunde mitgeteilt, welche den von Westheimer vorgeschlagenen Mechanismus der Zersetzung von Wasserstoffperoxyd durch Katalase ergänzen.

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Blood 'Contamination' of Liver Homogenates and the Liver Cathepsines

HOLZER et al.[1] suggested that the activity of some dehydrogenase enzymes and the concentration of some compounds (α-ketoglutarate, pyruvate) in liver homogenates might be influenced by the presence of blood in the liver. This suggested that the activity of other enzymes might also be influenced by blood. In the present report, which is part of a larger one, the possibility that blood might be a source of error in analysis of liver cathepsine and peptidase activity has been investigated.

Wistar rats, 150–200 g weight, were killed by decapitation, a blood sample was collected, and the liver rapidly excised. 1 ml of whole blood was diluted with 124 Vol of

[1] H. HOLZER, G. SEDLMAYER, and M. KIESE, Biochem. Z. *328*, 176 (1956).

7.5% polyvinylpyrrolidon (PVP) solution containing 0.03% saponine and homogenized (dilution factor 125 ×). 1 g of liver was diluted with 11.5 vol of PVP-saponine solution and homogenized (dilution factor 12.5 ×). Both suspensions were allowed to stand at room temperature for 30 min with occasional stirring; they were then centrifuged at 18000 *g* for 20 min and the supernatants preserved for analysis.

In appropriate aliquots of the supernatants the haemoglobin content was determined by the specific method of HAVEMAN et al.[2], using triethanolamine buffer at pH 7.5. With this method the increase in absorption (attributed specifically to haemoglobin) was measured at 546 mμ (or 570 mμ) after adding $K_3Fe(CN)_6$ (extinction 1) and then KCN (extinction 2). The differences in extinctions (ΔE) multiplied by the dilution factors represent the amount of haemoglobin in the undiluted original samples. The haemoglobin concentration in liver compared to that of the whole blood can be used to calculate an index of the contamination of the liver by blood.

The results of a representative experiment are seen in the Table.

The ΔE^{546} of 1 ml diluted whole blood sample was 0.200. Therefore the ΔE^{546} of 1 ml undiluted whole blood would be $0.200 \times 125 = 25$.

The ΔE^{546} of 1 ml diluted liver homogenate was 0.060. Therefore the ΔE^{546} of 1 g 'undiluted' liver (wet weight) would be $0.060 \times 12.5 = 0.75$.

Because 0.75 is 3% of 25, this would indicate that 3% of 1 ml whole blood (that is 30 μl) was found as a contaminant in 1 g of liver (wet weight), in this experiment.

Values of 1.5–5% (average 4.2%) have been obtained in a series of ten experiments. This variation appears to be dependent upon the circumstances of death.

After these, the analysis of the cathepsine and peptidase activity of liver, and of whole blood, was carried out. 1 g of liver, and 0.2 ml whole blood (many times the amount found as liver contaminant), was homogenized in 0.25 *M* saccharose or 0.1% of Triton X-100. Aliquots of the homogenates were mixed with buffer and substrate and the determinations carried out as described by RADEMAKER[3].

	After $K_3Fe(CN)_6$	After KCN	ΔE^{546}
$E^{546\ m\mu}$ blood	0.370	0.570	0.200
$E^{546\ m\mu}$ liver	0.360	0.420	0.060

The following enzymes were measured: (1) Cathepsine A activity with the specific substrate carbenzoxy *l*-glutamyline-*l*-tyrosine (0.04 *M*) at pH 5.1 (found as optimal) in citric acid-phosphate buffer. (2) Cathepsine B with 0.04 *M* benzoyl-*l*-argininamide at pH 5.5 in Michaelis buffer (veronal-acetate). (3) Cathepsine C with 0.04 *M* glycyl-*l*-phenyl-alanylamide at pH 5.4 in Michaelis buffer in which 0.02 *M* cystein was dissolved. (4) Carboxypeptidase with 0.04 *M* carbobenzoxy-glycyl-*l*-phenylalanine at pH 5 in citric acid-phosphate buffer. (5) Dipeptidase with 0.25 *M* diglycine at pH 7.7 in a phosphate buffer containing 0.002 *M* $CoCl_2$. (6) Tripeptidase with 0.16 *M* triglycine at pH 7.2 in phosphate buffer containing 0.002 *M* versene.

In experiments 2 and 3 ammonia was determined using the slightly modified method of VAN SLYKE and CULLEN[4]; the free acidic groups in experiments 1, 4, 5 and 6 were determined by the method of RADEMAKER and SOONS[5]. Mikrokjeldahl method was used for protein determinations.

With these methods, under the conditions specified, blood samples had no activity in the experiments 1, 5 and 6. In experiments 2, 3 and 4, the activity of 0.2 ml sample of whole blood was less than 5% of that for 1 g of liver (wet weight).

These findings, the presence of small amounts of blood and the insignificant activity of blood samples, indicate that the cathepsine and peptidase activity of rat liver homogenate is due to liver enzymes *per se*.

Zusammenfassung. Es wurde die Blutmenge in der Rattenleber sowie die Kathepsinaktivität der Leberhomogenate und des Blutes bestimmt. Unter den beschriebenen Bedingungen war die Kathepsinaktivität der Leberhomogenate nicht durch Blutkontamination beeinflusst.

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The Transulfuration of Sulfinates by Polysulfides

Organic polysulfides, produced either enzymically or by incubation of thiols with elementary sulfur, are known to transfer spontaneously an atom of sulfur to sulfinates[1–4]:

$$R\text{–SSH} + R'\text{–}SO_2H \longrightarrow R'\text{–}SO_2SH + R'\text{–}SO_2SH$$

This reaction is of biological importance since it may explain the production of thiosulfonates found in biological material as cysteine and cysteamine metabolites (see [3] for bibliography). Attempts to prepare the polysulfide analogue of cysteine (thiocysteine) demonstrated that this compound is unstable in aqueous solutions and is partially converted into cystine and inorganic polysulfides[1]:

$$2\ R\text{–SSH} \longrightarrow R\text{–SS–}R + H_2S_2$$

Instability in water solution is a general property of organic polysulfides and their life span is still unknown. Since inorganic polysulfides are expected to transulfurate sulfinates as well as organic polysulfides, the question rises whether the transulfurating agent is the organic or the inorganic polysulfide, produced through the decomposition of the former.

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