be worth while studying. In the case of the dichloro-dibromocompound, however, we have observed the evolution of hydrogen bromide both when melting the solid substance and when heating the substance in carbon tetrachloride solution to 200° C.

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Oxidation of Thiol Groups by Catalase

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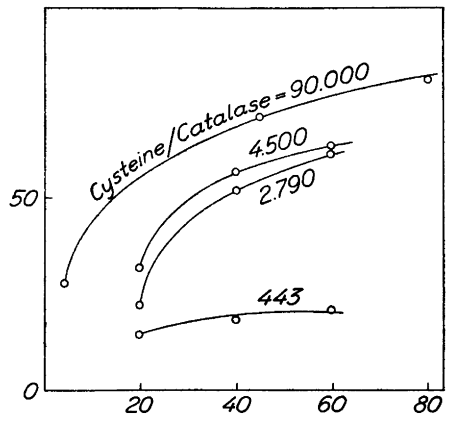

Fig. 1. Guinea-pig liver catalase. 20° C. Phosphate buffer M/100. Ordonates: percent decrease of the rate constant for the hydrogen peroxide decomposition as determined according to Bonnichsen, Chance and Theorell [6]. Abscissae: time in minutes of the incubation of the catalase solution with cysteine.

It is known that cytochrome-oxidase preparations in the presence of cytochrome-c and oxygen oxidize cysteine (Keilin [1]). We have found that the same reaction is effected by blood and liver catalase: oxygen is required, but neither cytochrome-c nor hydrogen peroxide.

The disappearance of the thiol groups was followed by titration of the thiol groups with the amperometric method of Kolthoff and Stricks [2] using a rotated platinum electrode, and the end product was identified by titrating the disulfide groups according to the same authors, after reduction with Na amalgam.

The rate of the catalytic action of catalase on cysteine oxidation is comparable to that of the system oxidase plus cytochrome-c: we obtained on a 10^{-3} *M* cysteine solution at pH 7 the same effect with 4.7×10^{-9} moles of catalase per ml as with a cytochrome-oxidase preparation whose Q_{02} was 212 per mg dry weight per hour (using 0.02 *M* succinate as substrate) with the addition of 1.25×10^{-8} moles of cytochrome-c per ml.

The catalytic effect of catalase on the oxidation of cysteine (and similarly on the oxidation of reduced glutathione and thioglycolic acid) is much higher than the hemin catalysis shown by Krebs [3]. No

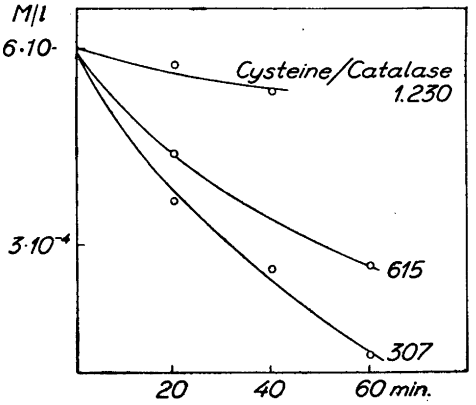

Fig. 2. Horse blood catalase. 20° C. Phosphate buffer M/100. Ordonates: cysteine concentration per liter: Abscissae: time in minutes.

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oxidation takes place if the catalase solution is boiled or if KCN is added in a concentration 1×10^{-3} *M*.

The rate of oxidation of cysteine in the presence of catalase is greatly influenced by the relative concentration of cysteine and catalase. At low ratios cysteine/catalase the rate of oxidation is high (see curves 2 and 3 in Fig. 1), about 9 moles of cysteine oxidized per mole of catalase per minute in the initial periode. At higher ratios cysteine/catalase, both the rate of oxidation of cysteine and the catalatic activity are inhibited. The former effect is seen in curve 1 of Fig. 1, where the rate reduced to 3 moles of cysteine oxidized per mole of catalase per minute. The latter effect has been known since Stern [4] and Waldschmidt-Leitz and collaborators [5], and appears clearly from Fig. 2.

A detailed report will shortly be published in this periodical, together with data on the spectroscopic changes of catalase in the presence of thiol groups.

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Preparation of Cytochrome c with the Aid of Paper Electrophoresis *

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The electrophoresis of proteins on filter paper was introduced by Durrum [1], Cremer and Tiselius [2], Turba and Enenkel [3] and Wieland [4]. We have found this method to be very useful for the purification of cytochrome c.

An apparatus described by Köiw, Wallenius and Grönwall [5] was used. The paper, "Munktell 20 350 g", was supplied from Grycksbo Paper Mills, Sweden. The size of the paper strips was 29 × 9 cm. 0.05 molar glycine-NaOH was used for pH 9.87 and 0.05 molar phosphate for pH 7.0. The voltage was 50—150 volts giving a current between 2.5 and 4 mA. The experiments were performed at room temperature. About 100 mg lyophilized cytochrome c in 1.0 ml of the buffer was applied across the middle of the buffer-wet paper by the aid of a Linderström-Lang pipette.

Sometimes a certain spreading of the preparation occurred when pressing the glass-plates together; however, it disappeared some hours after the electrophoresis had been started. The experiments lasted between 20 and 25 hours. After the experiment was finished the cytochrome fractions were extracted from the paper with 0.1 % ammonia.

Preparations of cytochrome c from beef and chicken hearts were made according to Keilin and Hartree [6]. The iron content of these starting materials was 0.296 % and 0.264 % respectively.

In the glycine buffer of pH 9.87 the cytochrome moved towards the cathode as was to be expected from the results obtained by free moving boundary electrophoresis. A brown impurity appeared from beef heart cytochrome, a yellow one from chicken heart cytochrome, both moving towards the anode. After about 20 hours the cytochrome was fully separated from the coloured impurities and was cut apart and extracted with ammonia. At pH 9.87 it was observed after some few hours that the cytochrome fraction moving towards the cathode had a tendency of dividing into a faster moving fraction of red-violet colour and a slower moving fraction of red-brown

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