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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colour, which were identified as ferro- and ferricytochrome. Iron analyses were made on the combined cytochrome fractions. The highest value obtained for beef heart cytochrome was 0.45 %, for chicken heart cytochrome 0.40 %. At pH 7.0, practically no ferrocytochrome c appeared. When mixing equal parts of ferro- and ferricytochrome obtained from the paper electrophoresis at pH 9.87 and subjecting the mixture to paper electrophoresis at pH 7.0, ferri- and ferrocytochrome separated again but now the former moved faster than the latter towards the cathode. Furthermore the ferricytochrome fraction from the electrophoresis at pH 9.87 was dissolved in glycine buffer of pH 2.7 and oxygen bubbled through for half an hour in order to ascertain complete oxidation. After lyophilization part of the preparation was dissolved in a buffer of pH 7.0 and part in a buffer of pH 9.8. At pH 7.0 the spot seemed to move uniformly as ferricytochrome, at pH 9.8 it separated again as ferri- and ferrocytochrome. This tendency to spontaneous reduction of cytochrome c at pH 9 — 10 has often been observed in this institute. It is interesting to notice that it occurs even with these highly purified preparations.

The separation of ferro- and ferricytochrome c in electrophoresis was to be expected from the titration experiments of Theorell and Åkeson [7], who found that the oxidized form has one basic equivalent per molecule more than the reduced form around pH 7, whereas the reduced form is more basic at pH-values above pH 9.6. The tendency of cytochrome c to separate into a reduced and an oxidized fraction between pH 9 and 10 was also observed when purifying cytochrome c on ion exchange resin [8]. The observed spontaneous reduction is not caused by impurities in the paper since the same happens in a test tube.

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