



Fig. 2. Density of colour as a function of thiocholine ester concentration.

×--× benzoylthiocholine bromide
 o--o acetylthiocholine iodide

Table 2. Chromogenic activity per thiocholine ester equivalent. Acetylthiocholine iodide = 100.

Compound	Chromogenic activity
Acetyl- α -methyl thiocholine bromide	100
Propionylthiocholine bromide	100
Butyrylthiocholine bromide	104
Valerylthiocholine bromide	104
Benzoylthiocholine bromide	143

from the calibration curves by the equation:

$$E = c \cdot d \cdot \epsilon$$

where c = mole/l standard solution and d = thickness of the layer in cm. The extinction coefficient was found to be 1.80×10^3 l mole⁻¹ cm⁻¹ for acetylthiocholine iodide and 2.55×10^3 l mole⁻¹ cm⁻¹ for benzoylthiocholine bromide. The chromogenic activity of the analysed thiocholine esters is seen from Table 2.

The colour of acetylthiocholine iodide was also determined in barbital buffer solution. The extinction obtained in this case was about 3 % lower than in phosphate buffer solution. Small amounts of protein, enough for determination of enzymatic hydrolysis, did not disturb the reactions.

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Reduction of some Haemo Proteins by Ultraviolet Light

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During some experiments with oxygen-free solutions of ferrimyoglobin exposed to CO it was noticed that the spectrum of ferrimyoglobin was not stable but slowly changed to that of MgbCO. Apparently ferrimyoglobin was reduced to ferromyoglobin, which subsequently reacted with CO. An examination of the actual conditions revealed that occasional irradiation

with ultraviolet light had caused the reduction. Some preliminary experiments have been performed on ferrimyoglobin, ferrihaemoglobin, ferricytochrome c, and catalase.

Methods. A low pressure water-conditioned mercury lamp was used as the UV source. The solutions were irradiated in a 1 cm Beckman quartz cell connected to the different gas tanks *via* a stopcock system. The specimen was introduced into the cuvette, the system evacuated and flushed with argon 4–5 times. In some experiments argon was replaced by CO in the last filling. The cuvette was kept at a distance of 7 cm from the arc. Na-phosphate buffer (pH 7.1, 0.05 M) was used as solvent.

The concentrations of the haem proteins were for myoglobin 70, haemoglobin 50, cytochrome c 50, and for catalase 20 μ M (2 μ M for experiments in the Soret band region). During the irradiations readings were taken at regular intervals at suitable wavelengths in a Beckman DU spectrophotometer.

Photoreduction of ferrimyoglobin. After ten minutes 50 % and after 25 min. 90 % of the ferri-Mgb had been reduced and converted to MgbCO, as calculated from the light absorptions at 580 $m\mu$. The addition of dithionite raised the optical density at 580 $m\mu$ to the value calculated for complete conversion to MgbCO. If no CO was present a haemochromogene type spectrum was obtained with maxima corresponding to those of MgbO₂. The very low concentration of oxygen decreased during irradiation as determined polarographically. This was probably due to the fact that the minute amounts of oxygen present at the beginning of the experiments were gradually taken up by Mgb. There did not seem to be any liberation of oxygen during irradiation. The addition of catalase did not influence the reaction velocity.

Photoreduction of ferrihaemoglobin. Half reduction and conversion to HbCO was obtained after 10 min. and 80 % HbCO after 20 min. Addition of dithionite gave a 7 % too low value, probably due to destruction. Irradiated Hb showed, within

the errors of the method, the same affinity for CO as Hb, reduced with dithionite.

Photoreduction of ferricytochrome c. No CO was present in these experiments. Ferricytochrome was half reduced after 12 minutes and 75 % reduced after 20 min. (followed at 549.5 $m\mu$). In this reaction cytochrome c seemed to be highly sensitive even to traces of oxygen. It was in no case possible to obtain the expected light absorption at 549.5 $m\mu$ by reduction with dithionite of an irradiated solution. Between 10–15 % of the total cytochrome seemed to have been destroyed. The maxima of the absorption bands of photo-reduced cytochrome c were found at 549.5 $m\mu$ and 520.5 $m\mu$, exactly in the same positions as those of cytochrome c reduced with dithionite.

Photoreduction of catalase. During the irradiation of liver catalase a change in the absorption spectrum could be noticed and after 45 min. weak absorption bands at 550 $m\mu$ and 580 $m\mu$ could be observed. In the absence of CO no spectral change was seen. In the presence of dithionite (which does not reduce catalase but acts as an oxygen remover) and without CO a broad absorption band at about 560 $m\mu$ appeared. Upon the addition of CO to this solution the same spectrum appeared as obtained without dithionite but with CO initially present. During irradiation in the presence of dithionite the Soret band maximum at 405 $m\mu$ shifted to 422 $m\mu$ and became somewhat reduced in height. In this case CO partly reversed the shift, from 422 to 418 $m\mu$. The changes of the catalase caused by the irradiation seemed to be fully reversible. Contact with air restored the initial density value at 575 $m\mu$. The activities of a specimen¹ before and after its irradiation with UV light were the same.

A detailed report of these experiments will be published shortly. The investigations are being continued.

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