Estimation of Microquantities of Thiocholine Esters

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In order to determine the spontaneous hydrolysis and the initial enzymatic hydrolysis of several thiocholine esters an exact method for their quantitative analysis was sought for. The method developed by Hestrin [1] for the estimation of choline esters and other carboxylic acid derivatives was tried and was found to be applicable to the thiocholine esters as well.

This method makes use of the fact that choline esters react quantitatively with hydroxylamine in alkaline solution according to the following scheme

$$RCOOR' + H_2NOH \rightarrow RCONHOH + R'OH$$

The resulting hydroxamic acid can be determined by the coloured complex with ferric chloride in acid solution.

The thiocholine esters used in this investigation, except acetylthiocholine iodide, were synthesized by Hansen [2]. The following esters were used:

acetylthiocholine iodide
acetyl-α-methylthiocholine bromide
acetyl-β-methylthiocholine bromide
propionylthiocholine bromide
butyrylthiocholine bromide
valerylthiocholine bromide
benzoylthiocholine bromide

Experimental. The same reagents as in Hestrin's method [1] for carboxylic acid derivatives were used.

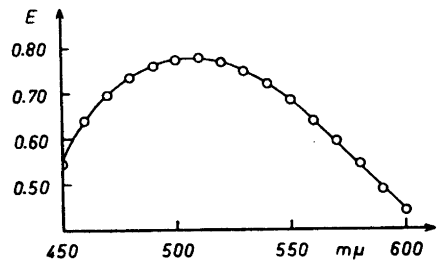

Fig. 1. Absorption curve of the ironhydroxamic acid complex.

Standard solutions: 0.5—10.0 mmole thiocholine ester in Clark and Lubs' [3] phosphate buffer solution at pH 8.00 or in barbital buffer solution [4] at pH 8.07 were used.

The buffer solutions were allowed to reach the thermostat temperature in a volumetric flask. The thiocholine esters were weighed excluding moisture as much as possible and rapidly dissolved in the buffer solution. 1.00 ml of the samples was immediately withdrawn and analysed according to the method developed by Hestrin [1]. The colour was measured in a 10 mm cell with a Beckman B spectrophotometer.

Table 1. Extinction of different concentrations of acetylthiocholine iodide in phosphate buffer solution after varying reaction time with hydroxylamine.

Acetylthiocholine iodide mmole/l	*E* time in min. 1	2	3
10	1.800	1.815	1.807
8	1.441	1.439	1.433
6	1.080	1.072	1.075
4	0.720	0.720	0.741
2	0.365	0.366	0.369
1	0.184	0.186	0.190
0.5	0.093	0.095	0.097

Results. The absorption maximum for the coloured complex was found to be at 510 mμ as shown in Fig. 1.

The reaction with hydroxylamine was carried out for 1, 2 and 3 minutes at room temperature. The extinction appears to be independent of time after 1 minute as can be seen from Table 1. The variation of optical density with ester concentration is shown in Fig. 2 for acetylthiocholine iodide and benzoylthiocholine bromide. It appears from these curves that Lambert Beer's law is obeyed. The lower limit of measurement is about 0.05×10^{-3} mmole of final solution. The upper limit exceeds 2×10^{-3} mmole of final solution. At concentrations of 6×10^{-3} mmole of final solution and above, a precipitate, which is very difficult to centrifuge off, was observed. The precipitate may be sulfur.

The molar extinction coefficient, ε, of the two thiocholine esters was calculated

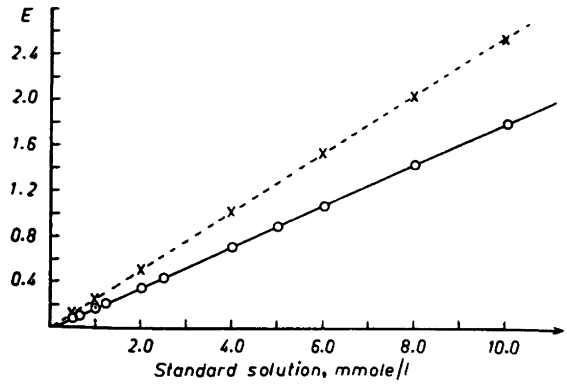

Fig. 2. Density of colour as a function of thiocholine ester concentration.

×--× *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:

$$E = c \cdot d \cdot \varepsilon$$

where c = mole/l standard solution and d = thickness of the layer in cm. The extinction coefficient was found to be 1.80×10^2 l mole^{-1} cm^{-1} for acetylthiocholine iodide and 2.55×10^2 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