Summary

The enzymatic hydrolysis of propionylcholine (PrCh), acetylthiocholine (AcThCh), and butyrylthiocholine (BuThCh), by extracts of the muscle rectus abdominis, was determined. Inhibition of this hydrolysis by D.F.P. and 3318 CT [bis(pipéridinométhylcoumaranyl-5)cétone diméthiodide] – utilized over a range of concentrations covering both specific and non-specific concentrations – showed that PrCh is hydrolyzed by an acetylcholinesterase (70%) and an Xcholinesterase (30%), AcThCh by the AcChE (70%), the XChE (15%) and a thioesterase (15%) and BuThCh by the XChE (70%) and a thioesterase (30%).

A Colorimetric Determination of Aminooxy Acids

In the course of preparative work on canaline[1], $NH_2OCH_2CH_2CH(NH_2)COOH$, it became desirable to develop a method for its quantitative determination.

KITAGAWA[2] observed that canaline gives an orangered colour with alkaline picrate. This colour reaction (Jaffe's test) was attributed by him to the presence of the free amino-oxy group in the molecule; it is also given by other free α-hydroxylamino compounds.

This qualitative reaction was applied to develop a colorimetric method for the determination of canaline, of amino-oxy-acetic acid, and of hydroxylamine.

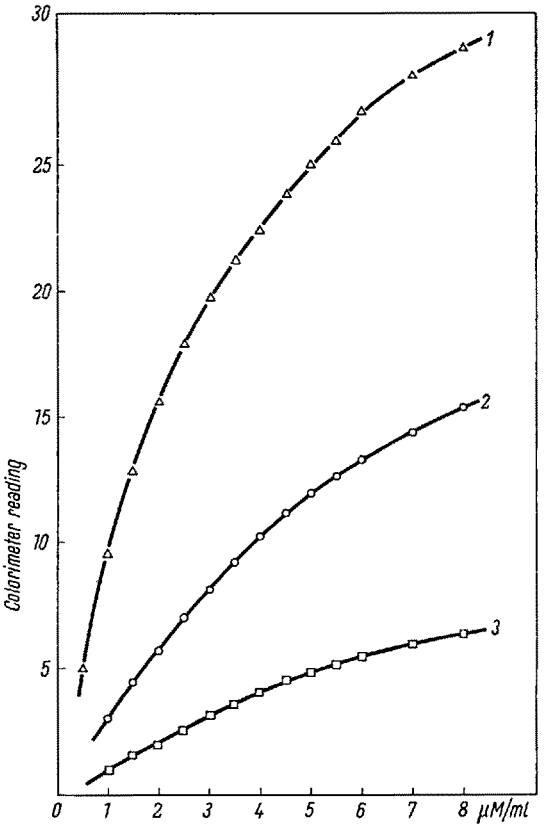
A Fisher electrophotometer (AC model) was used at 525 mμ, and curves showing the relation between optical density and concentration were constructed to serve for the quantitative determination of the amino-oxy acids in question.

The development of the colour, after addition of saturated aqueous picric acid and 1% sodium hydroxide to the water solution of the amino-oxy compound, is gradual, reaching maximal intensity after 5–10 min. At this point the determination has to be carried out immediately. The best proportionality obtained for canaline was in the range of 1 to 4 micromoles (μM) per ml, for amino-oxy-acetic acid between 1·5 and 5·5 μM per ml, and for hydroxylamine between 0·5 and 3 μM per ml.

Presence of sodium chloride was found to have no appreciable effect on the colorimeter readings, thus allowing estimation of hydrochloric acid salts of the amino-oxy acids. Solutions of the latter, kept for several days, gave very similar curves. The influence of exposure to light was also negligible.

The three curves, showing the relation between optical density of the alkaline picrates of canaline, of amino-oxy-acetic acid, and of hydroxylamine concentrations respectively are given in the Figure.

Calibration.—To 1 ml of the diluted solution containing canaline, amino-oxy acetic acid, or hydroxylamine respectively (final concentration 0·5 to 8 μM per ml) was added 1 ml of saturated aqueous picric acid and then 1 ml of 1% sodium hydroxide and 2 ml of water. After mixing the contents, the tube was allowed to stand for 5 min. After that it was introduced into the Fisher photoelectric colorimeter, equipped with a 525 mμ filter, and compared with the solution which served as the blank control and contained 3 ml of water, 1 ml of saturated aqueous picric acid, and 1 ml of 1% sodium hydroxide. Further readings were taken after the seventh and ninth minute.



Optical density readings at various concentrations of the aminooxy compounds.

1: hydroxylamine, 2: canaline, 3: aminooxy-acetic acid.

Series of colorimeter tubes containing the appropriate amounts of the amino-oxy compound to be determined were set up and compared with the blank control. The average values of the three readings for each concentration, taken at 5, 7, and 9 min respectively, served as basis for the construction of the curves. Crystalline DL-canaline[1] m.p. 190° C, amino-oxy acetic acid hemihydrochloride[3] m.p. 154° C, and commercially available pure hydroxylamine hydrochloride (May and Baker, London), were used.

Procedure.—The amino-oxy compound to be determined, diluted to give a final concentration of seven to one half μM, was transferred to the small colorimeter tube, reagent solutions and water were added, and the electrophotometer scale reading determined after comparing with the blank control, all as given under Calibration. The content of the compound was determined by reference to the respective calibration curve.

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Résumé

Une méthode colorimétrique pour la détermination quantitative des acides oxyaminés basée sur la réaction rouge-orange caractéristique (réaction de Jaffe) a été mise au point.

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