FLAGYL AND REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE

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1. Introduction

Flagyl [1] is the trade name of metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, the drug most commonly employed in the treatment of infections of the vagina by *Trichomonas vaginalis*. Recently it has been widely used against entamoebal infections. Flagyl has also been demonstrated to produce aversion to ethanol, and was suggested as a repressant of compulsive consumption of alcohol [2].

It was reported in 1968 by Fried and Fried [3] that the 340 nm absorption of NADH is titrated away, in a purely chemical reaction, by increasing concentrations of Flagyl in the absence of any other reactants. They suggested that this was a result of Flagyl acting as a relatively non-specific electron trap, thus causing the oxidation of NADH upon mixing. In view of the potential importance of this reaction we have attempted to repeat these experiments. This communication gives the results of such an investigation, which clearly demonstrate that the reported reaction between Flagyl and NADH does not occur.

2. Materials and methods

Flagyl was obtained from May and Baker Ltd., Dagenham, Essex, England. NADH was supplied by Boehringer Co. (London) Ltd. A Cary 14 and a Perkin Elmer 356 were used for the spectrophotometric measurements.

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3. Results

Repeating the experiment of Fried and Fried using the Perkin Elmer 356, in the classical split beam mode, produced the results shown in fig. 1, thus giving strong support for the reaction of NADH with Flagyl. However when the same solutions were measured using 2 mm pathlength cells, instead of 1 cm pathlength cells, thus lowering the background absorbance of Flagyl, no more than a very slight decrease in 340 nm absorbance was observed (fig. 2).

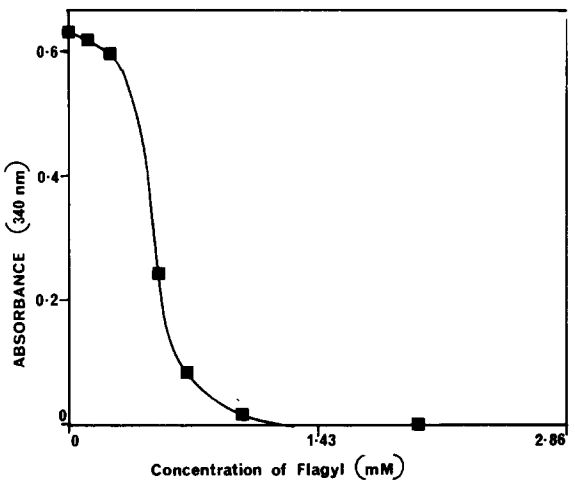

Fig. 1. The 'apparent' oxidation of NADH by Flagyl. Measured in 1.0 cm pathlength cuvettes containing 0.34 μmoles NADH, Flagyl and phosphate buffer (0.05 I, pH 7.2). Final volume 2.8 ml. Measured against blanks containing the same concentrations of Flagyl in phosphate buffer. Perkin Elmer 356 spectrophotometer.

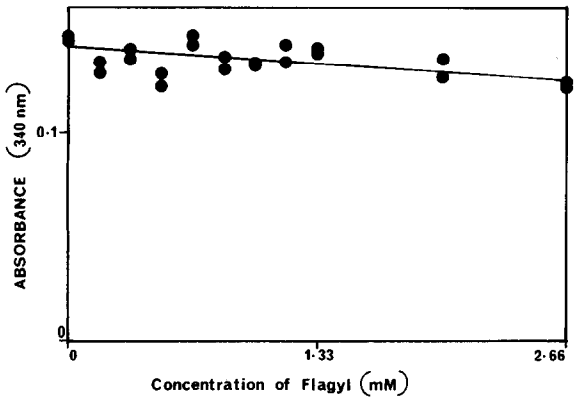

Fig. 2. The true effect of Flagyl on NADH. Measured in 2 mm pathlength cuvettes from a reaction mixture containing 0.32 μmoles NADH, Flagyl and phosphate buffer (0.05 I, pH 7.2). Final volume 3.0 ml. Measured against blanks containing the same concentrations of Flagyl in phosphate buffer. Cary 14 spectrophotometer.

An explanation of both Fried and Fried's results and those given above for work with 1 cm pathlength cells was clearly demonstrated when this experiment was repeated but, instead of simply measuring the 340 nm absorbance, the whole spectrum, from 400 nm to 240 nm, was scanned. The results (fig. 3) from this experiment indicated that the apparent progressive oxidation of NADH by increasing concentrations of Flagyl was caused by failure of the spectrophotometer to measure the true absorbance of the reduced nucleotide in the presence of such a high background Flagyl absorbance.

The lack of chemical reaction between NADH and Flagyl was confirmed beyond all doubt by the use of split compartment cuvettes. The spectrum before and after mixing of the two solutions was identical. It was also demonstrated that the 'titration effect' could be achieved although the NADH and Flagyl solutions were saparated by a quartz wall.

Further confirmatory experiments included one in which the 320 nm absorbance of a solution of Flagyl was shown to completely disappear upon addition of a strong Flagyl solution. Thus it appeared that Flagyl had titrated away its own 320 nm absorbance!

4. Discussion

It has been shown that no spectrophotometrically observable chemical reaction occurs between Flagyl

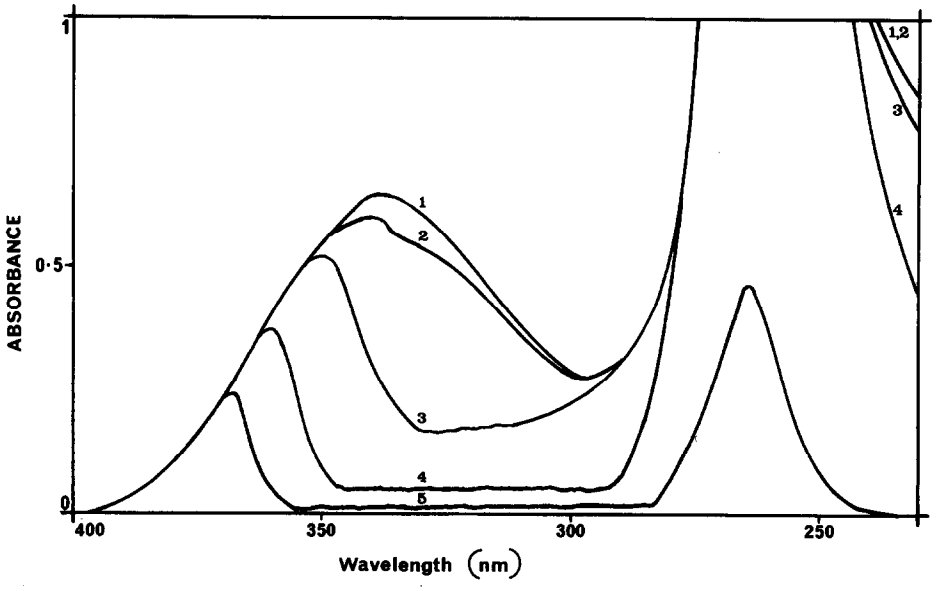

Fig. 3. The spectrum of NADH measured in the presence of various concentrations of Flagyl. Measured in 1.0 cm pathlength cuvettes containing 0.34 μmoles NADH, Flagyl and phosphate buffer (0.05 I, pH 7.2). Final volume 2.8 ml. Content of Flagyl: (1) zero; (2) 0.7 μmoles; (3) 1.4 μmoles; (4) 2.8 μmoles; (5) 5.6 μmoles. Perkin Elmer 356 spectrophotometer.

and NADH. Flagyl does not act as an electron sink, and hence oxidant, for the reduced nucleotide. This does not however make any the less valid Fried and Fried's conclusion that a lot of work involving inhibition of dehydrogenases by Flagyl may be untrustworthy; the apparent inhibitions may be due simply to spectrophotometric artefacts.

Acknowledgements

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References

[1] May and Baker Ltd., Dagenham, Essex, England.
[2] Taylor, J. A. T. (1964) Bull. Los. Ang. neurol. Soc. 29, 158.
[3] Fried, R., and Fried, L. W. (1968) Experientia 24, 56.