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SHORT COMMUNICATIONS

In vitro oxidation of DPNH by free radicals of chlorpromazine

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IT WAS reported previously that chlorpromazine is an effective inhibitor of various dehydrogenases.[1, 2] In previous papers we have shown that chlorpromazine, if oxidized partially or irradiated, directly inhibits PGAD without incubation.[3, 4]

In order to investigate the mechanism of this inhibition, chlorpromazine was partially oxidized with ceric sulphate. If an equivalent amount of DPNH*, TPNH or $Na_2S_2O_4$ was next added, the wine red colour of the free radical disappeared, as shown in the absorption spectrum of Fig. 1.

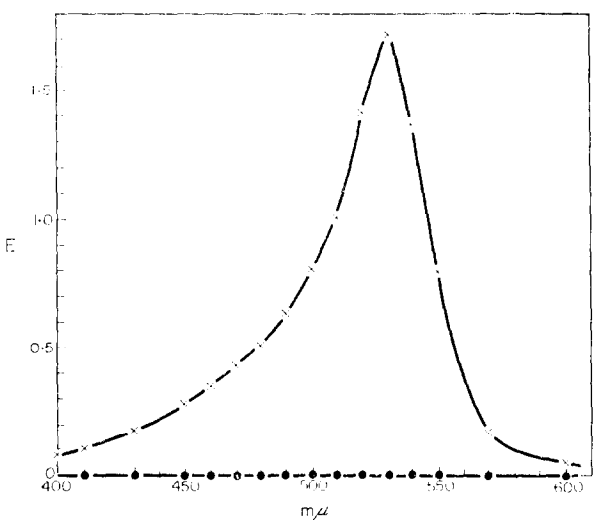

FIG. 1. ×——× $2{\cdot}5 \times 10^{-3}$M chlorpromazine + 3×10^{-3}M $Ce(SO_4)_2$.
·——· $2{\cdot}5 \times 10^{-3}$M chlorpromazine + 3×10^{-3}M $Ce(SO_4)_2$ + 3×10^{-3}M DPNH, TPNH or $Na_2S_2O_4$.

After irradiating a 10 mg/ml chlorpromazine solution for 32 hr (quartz lamp, 3 A; distance, 20 cm) a brownish red colour appeared which, after addition of the same reducing agents, turned to a faint yellow (Fig. 2).

Studying the absorption maximum of DPNH at 340 mμ, a decrease of its value was observed after addition of irradiated chlorpromazine but not of chlorpromazine (Table 1).

Measuring the ultra-violet absorption spectrum of the free radical, produced with ceric sulphate, after addition of DPNH or TPNH, some diminution of the absorption maximum at 275 mμ was

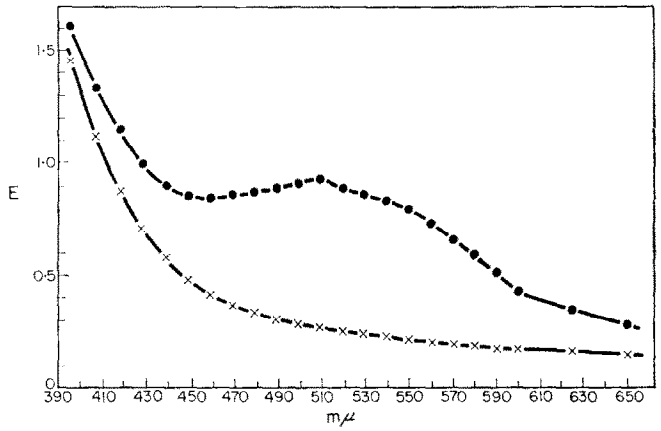

FIG. 2. ·——· 2·5 × 10 ^{3}M irradiated chlorpromazine.
+——+ 2·5 × 10^{-3}M irradiated chlorpromazine + 3 × 10 ^{3}M DPNH, TPNH or $Na_2S_2O_4$.

TABLE 1. DECREASE OF THE ABSORPTION MAXIMUM OF DPNH AT 340 Mμ AFTER ADDITION OF IRRADIATED CHLORPROMAZINE

	E
8·5 × 10 ^{5}M chlorpromazine	0·12
8·5 × 10^{-5}M irradiated chlorpromazine	0·175
8·5 × 10 ^{5}M DPNH	0·205
8·5 × 10^{-5}M chlorpromazine + 8·5 × 10 ^{5}M DPNH	0·33
8·5 × 10 ^{5}M irradiated chlorpromazine + 8·5 × 10^{-5}M DPNH	0·18

observed (Fig. 3). Treating the free radical, after addition of DPNH or TPNH, according to the extraction method of Salzman and Brodie,[5] it turned out to be a mixture of chlorpromazine and chlorpromazine sulphoxide. However, repeating the extraction method without addition of DPNH or TPNH, i.e. with the free radical alone, appearance of chlorpromazine and chlorpromazine sulphoxide was also observed.

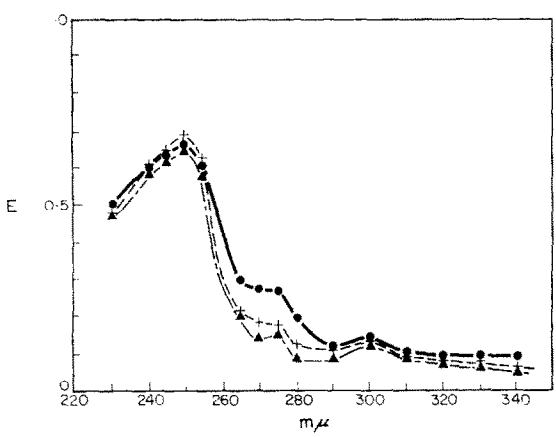

FIG. 3. ·——· 2·5 × 10^{-5}M chlorpromazine + 3 × 10^{-5}M $Ce(SO_4)_2$.
+——+ 2·5 × 10^{-5}M chlorpromazine + 3 × 10^{-5}M $Ce(SO_4)_2$ + 3 × 10^{-5}M TPNH.
A——A 2·5 × 10^{-5}M chlorpromazine + 3 × 10^{-5}M $Ce(SO_4)_2$ + 3 × 10^{-5}M DPNH.

Borg and Cotzias[6] also mention the spontaneous slow transformation of the free semiquinone radical into chlorpromazine and chlorpromazine sulphoxide. A similar effect was observed with DPNH by Beinert and Sands;[7] in the presence of DPNH cytochrome *c* reductase, addition of DPNH decreased the free radical concentration of flavin semiquinones.

When chlorpromazine was irradiated in the presence of 0·02 M EDTA, the change of colour did not proceed all the way to brownish red, but stopped at the occurrence of a blue precipitate.

From all this we conclude that some metal traces must have a catalytic role in the oxidation reaction.

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* Abbreviations used, DPNH, reduced diphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; PGAD, D-glyceraldehyde-3-phosphate dehydrogenase.
Chlorpromazine, DPNH and TPNH were gifts from Specia (Paris) and Light (Colnbrook).

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The effect of some nucleotoxic agents on urinary excretion of 5-hydroxyindolacetic acid in rats

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IT HAS been found that X-irradiation increases the urinary excretion of 5-hydroxyindolacetic acid (5-HIAA) in rats.[1, 2] Since nucleotoxic drugs show certain similarities with ionizing radiation, the influence of these substances on the excretion of 5-HIAA was examined. According to Dustin,[3] the nucleotoxic drugs can be divided into radiomimetic and spindle poisons. In our investigation, nitrogen mustard represented the first and urethane, the second group. For comparison, another hypnotic drug, chloral hydrate, which is not a nucleotoxic agent, was also tested.

Albino rats of both sexes, from our Institute stock, weighing 150–250 g were used. Groups of four rats were placed in glass metabolic cages and urine was collected under glacial acetic acid during the 3 days before and the 3 days after treatment. The rats were allowed water *ad libitum* but food was given them during only 1 hr daily. The determinations of 5-HIAA in 24-hr urine specimens were made using the simplified method described by Dalgliesh[4] because the concentration of interfering keto acids was negligible. The colorimetric estimation was performed with a C. Zeiss spectrophotometer, model VSU 1. Nitrogen mustard (N-methyl-bis-(chloroethyl) amine, Antimit "Pliva") was administered intraperitoneally; urethane and chloral hydrate, subcutaneously. The doses of the tested drugs correspond very nearly to the lethal ones in order to reproduce the conditions of X-irradiation experiments.