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.

TABLE 1. THE URINARY EXCRETION OF 5-HYDROXYINDOLACETIC ACID IN RATS (μg/100 g BODY WEIGHT PER HR) BEFORE AND AFTER NITROGEN MUSTARD, URETHANE AND CHLORAL HYDRATE (SEE TEXT)

	Groups of 4 rats	Before treatment		After treatment		
		3rd day	2nd day	1st day	1st day	2nd day	3rd day
Nitrogen mustard 3 mg/kg ip	6	0·70 ± 0·03*	0·80 ± 0·08	0·60 ± 0·03	0·70 ± 0·07	0·87 ± 0·06†	0·32 ± 0·04
Saline 5 ml/kg	6	0·63 ± 0·04	0·70 ± 0·06	0·61 ± 0·05	0·59 ± 0·02	0·59 ± 0·01	0·55 ± 0·10
Urethane 1500 mg/kg sc	6	0·64 ± 0·10	0·78 ± 0·05	0·72 ± 0·05	0·61 ± 0·04	0·92 ± 0·05†	0·86 ± 0·06
Saline 5 ml/kg	6	0·71 ± 0·06	0·62 ± 0·07	0·71 ± 0·05	0·64 ± 0·04	0·65 ± 0·07	0·76 ± 0·09
Chloral hydrate 500 mg/kg sc	6	0·76 ± 0·07	0·77 ± 0·07	0·71 ± 0·06	0·73 ± 0·07	0·75 ± 0·08	0·74 ± 0·06
Saline 5 ml/kg	6	0·71 ± 0·06	0·62 ± 0·07	0·71 ± 0·05	0·64 ± 0·04	0·65 ± 0·07	0·76 ± 0·09

* Standard error of the mean.
† Significant difference as compared with control at 5 per cent level (*t* test).

The experimental data are summarized in the Table 1. It may be noted that nitrogen mustard and urethane produce a significant increase in 5-HIAA urinary excretion on the second day after injection. Chloral hydrate showed no effect. Therefore it seems that only nucleotoxic agents increase the excretion of 5-HIAA. In this respect there is no difference between the so-called radiomimetics and spindle poisons. Another spindle poison colchicin was shown by Marks and Sorgen[5] to produce degranulation of enterochromaffin cells, a result which is consistent with the present observations. It seems that these cells are also sensitive to both ionizing radiation and nucleotoxic agents. The time of maximal urinary excretion of 5-HIAA is, however, different in each case occurring on the first day after treatment for ionizing radiation, but on the second day for nucleotoxic agents.

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Some properties of isozymes of brain acetylcholinesterase *

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It has been reported that the acetylcholinesterase of human brain consists of three isozymes when it is separated under the conditions described.[1] Since a vast literature exists on the biochemical properties and physiological function of this enzyme, it was thought that a comparison of some of the properties of the acetylcholinesterase isozymes might provide some insight into the nature of their differences. At the present time the nature and significance of multiple forms of a given enzyme are virtually unknown, and such a study could provide some data on this problem.

Starch-gel electrophoresis was performed according to Smithies.[2] After the starch-gel separation had been completed, one channel was cut from the gel and the enzyme localized by methods already described.[3] This was matched against the remaining gel, and the areas of enzymatic activity were dissected from the starch block.

Because of the proximity of the two fastest moving isozymes to each other, their isolation as individual components was not feasible, and they were treated as one band (band 2). Attempts at extracting the enzyme from the starch-gel by various techniques yielded recoveries of 10 to 20 per cent of the enzymatic activity. Consequently, the starch-gel was homogenized with an equal volume of distilled water to which 2% gum acacia was added to stabilize the enzyme. This was dialyzed overnight against 0·9% saline. No loss in enzymatic activity was noted during this procedure. Aliquots of the slurry were used as the enzyme source, and acetylcholinesterase activity was determined by a micro-titrimetric method[4] using acetylcholine iodide as a substrate in the presence of 0·01 M $MgCl_2$. No activity was discernible with butyrylcholine iodide as a substrate. All values were corrected for spontaneous hydrolysis. The determinations of the substrate optimum, Km values, as well as the inhibitor data were obtained at pH 7·5, and 37°. The Km values were obtained by treatment of the data according to Lineweaver and Burk[5], using the values on the low concentration side of the curve. The isozyme proximal to the origin is designated as band 1, and the distal isozymes as band 2. Human caudate nucleus and putamen were again used as an enzyme source. All values reported are the averages of three to six determinations.

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